Varicocele and Male Infertility

A Complete Guide

Sandro C. Esteves
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Editors
Sandro C. Esteves, MD, PhD
ANDROFERT
Andrology & Human
Reproduction Clinic
Campinas, SP
Brazil

Ahmad Majzoub, MD, MBChB, CABU
Department of Urology
Hamad Medical Corporation
Weill Cornell Medicine Qatar
Doha
Qatar

Chak-Lam Cho, MD, FRCS-Ed (Urol)
S.H. Ho Urology Centre
Department of Surgery
Prince of Wales Hospital
The Chinese University of Hong Kong
Hong Kong
China

Ashok Agarwal, PhD, HCLD (ABB)
Department of Urology
Cleveland Clinic
Cleveland, OH
USA

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To my late father, Waldemar Esteves, for instilling the virtues of integrity, perseverance, and enthusiasm. To my wife, Fabiola, and sons, Alexandre and Catarina, for their devotion, love, and support. To late Professor Nelson Rodrigues Netto Jr. (UNICAMP), late Professor Anthony Thomas (Cleveland Clinic), and Professor Ashok Agarwal (Cleveland Clinic) for their guidance and support and for being the giants who take their apprentices by their hands and teach them how to climb and, once on their shoulders, show what to see—helping us to see better and further. –Sandro C. Esteves

I would like to dedicate this book to my wife, Amy, and our three sons, Maurice, Liam, and Xavier, for their love and support; to my mentors, Dr. Kwan-Lun Ho, Dr. In-Chak Law (Kwong Wah Hospital), and Prof. Ashok Agarwal (Cleveland Clinic), for enriching my life with their guidance and support; to my colleague, Dr. Ringo Wing-Hong Chu, for our friendship; and to our patients for inspiring us the art and science of medicine. –Chak-Lam Cho

Life has taught us that hard work eventually pays off. This book is a solid evidence of the outcome of devotion and determination. Nonetheless, no man is capable of achieving any accomplishment without the love and support of those who surround him. I am proud to dedicate this book to my beautiful wife, Zeinab, for her unconditional love and continuous encouragement to follow my dreams. To the greatest muse who always shed a light on dreary roads, my darling angels, Sarah and Tala.

I am ever so grateful to Prof. Ashok Agarwal for his utter and absolute belief in me and for the opportunities he made possible. He was the driving force to success at a crucial turning point in my life. Last but not least, my sincere gratitude goes to Dr. Edmund Sabanegh, a wonderful mentor and a role model of total dedication to a promising career.

–Ahmad Majzoub

To my late father, Professor RC Aggarwal, for instilling the virtues of honesty, dedication, and hard work. To my wife, Meenu, and sons, Rishi and Neil-Yogi, for their unconditional love and support. To Professor Kevin Loughlin (Harvard Medical School), late Professor Anthony Thomas (Cleveland Clinic), and Professor Edmund Sabanegh (Cleveland Clinic) for their friendship, guidance, and support and for making an indelible positive impression on my life. –Ashok Agarwal

Foreword

Varicocele and Male Infertility: A Complete Guide is a comprehensive reference book related to the diagnosis, treatment, and pathophysiology of varicoceles. The reader will find that its 58 chapters cover an extensive time period from antiquity to the present day. Furthermore, the topics include material related to the diagnosis of these lesions, early surgery that was used for the treatment of pain, description of more refined surgery that was introduced in cases of male infertility, and new aspects of the pathophysiology of varicoceles as reported on humans and laboratory animals.

The historical chapters indicate that varicoceles were recognized during the time of the Roman Empire, because these lesions were often visible and palpable and caused pain. Surprisingly, the earliest reported surgery to correct a varicocele was published in the first century AD. Although the anatomy was poorly understood, the rudimentary scrotal surgery seemed adequate to provide pain relief for patients. Therefore, this time frame was the so-called Pain Era, and it lasted until the mid-twentieth century before varicocele surgery was first utilized for the treatment of male infertility.

With the start of the "Fertility Era," the interest in varicoceles increased dramatically, because many more patients were seen with varicoceles and infertility than pain alone. At the start of this new era, various surgical procedures were introduced, such as high inguinal surgery, microsurgery in the inguinal and subinguinal areas, laparoscopy, and robotic surgery. As a result of these techniques, there were fewer postop hydroceles and fewer injuries to the testicular arteries. In addition, other techniques were introduced for adolescents with varicoceles in an attempt to prevent future infertility. Although these procedures were simplified by the utilization of venography, venous occlusion, and sclerosis, this topic is still being debated, and aspects of the debate are included in the specific chapters of the book.

In the mid-twentieth century, the entire field of male infertility was emerging as it became part of the specialty of urology. During this time period, molecular biologists began to investigate and uncover new findings related to the pathophysiology of varicoceles and infertility. For example, the practical application of clinical venography clarified that there was reflux in the internal spermatic veins due to the absence of valves, and this reflux produced increased scrotal heat and pressures in men with varicoceles. However, recent studies revealed that the pressure within the reflexive veins released reactive oxygen species that affected sperm function. Other recent findings revealed evidence of increased sperm DNA damage among men with varicoceles, and

other studies reported that correction of a varicocele may improve Leydig cell function to increase serum testosterone. Recently, some studies reported that a varicocele may influence the outcome of IVF/ICSI and that alterations in the seminal proteomics may affect sperm production within the testes. Overall, many biological discoveries related to varicoceles have been reported in the last 50 years among men with varicoceles and in laboratory animal models, and these findings have been reported in the book chapters.

In summary, those who read and study this book will have many rewards. The information within the book will serve the readers well in the clinical practice and in the laboratory. Furthermore, the readers will recognize that the editors have selected an outstanding group of investigators and clinicians to develop the chapters for this comprehensive reference book. I am honored to have been a contributor, and I am sure that all of the other contributing authors feel the same way. As the reader proceeds through the chapters, I believe that they will refer to the book frequently. In the future, perhaps some of the readers will be stimulated to become authors themselves. In any case, enjoy the book!

Joel L. Marmar, MD
Honorary Staff (retired)
Cooper University Hospital
Camden, NJ, USA

Professor of Surgery/Urology (retired)
Cooper Medical School of Rowan University
Camden, NJ, USA

Preface

Varicocele, from the Latin word *varix* (dilated vein) and the Greek word *kele* (tumor), has been recognized as a clinical entity for over a century. This condition is the leading cause of male infertility, as it can impair spermatogenesis through several distinct pathophysiological mechanisms. However, despite over 2000 scholarly articles published since its first report in the eighteenth century, varicocele still elicits debate among scientists and clinicians. The main reason stems from the fact that not all affected men have decreased fertility and impaired gonadal function, thus making varicocele the most debatable issue in the field of urology, andrology, and reproductive medicine.

The field of reproductive medicine has evolved dramatically over the past 40 years. The advent of ICSI in 1992 ignited a big leap forward in the rapid development of assisted reproductive techniques, while little attention has been given to the evaluation of infertile men. We have witnessed a return of interest in male infertility in recent decades driven by the concept that varicocele treatment does not only enhance sperm quality and improve natural pregnancy outcomes but also pose a positive impact on outcomes of assisted reproduction. Thus, it is the prime time to summarize the pertinent background and latest advances in this ever-changing field.

Varicocele and Male Infertility is written by 105 internationally recognized experts from 13 countries and 5 continents and organized in 7 sections and 58 chapters. Part I, dealing with the origin and pathophysiology of varicocele, is encapsulated in 7 chapters; Part II on the clinical evaluation of varicocele is described in 7 elegantly written chapters; Part III on varicocele therapy is dealt in 15 well-elaborated chapters; Part IV has 10 chapters dealing with the controversies surrounding varicocele; Part V on varicocele debate covers both pro and con positions in 8 impressive chapters; Part VI with 7 chapters covers a variety of clinical case scenarios on varicocele; and, lastly, Part VII with clinical practice guidelines is well covered in 4 articles.

Our book is intended to provide a thoughtful and comprehensive view of the significance of varicocele and its impact in male infertility from a multitude of angles. Controversies and the reasons behind these arguments about varicocele were illustrated to all healthcare professionals and researchers by compiling the work from a group of distinguished, internationally recognized contributors. Essential to any practicing urologist, reproductive specialist, and researcher involved in andrology and reproductive medicine, *Varicocele and Male Infertility* is the first of its kind. Filled with art diagrams,

photographs, and tables, this book is an invaluable resource for learning and teaching. Each chapter includes a section of "key points" to allow rapid acquisition of prominent information. Moreover, multiple-choice questions are provided to test the knowledge of the readers. It is an exciting time to be involved in the treatment of infertility. We genuinely hope that this volume will stimulate your interest and enrich your clinical practice in the management of subfertile men with varicocele.

We, the editors, are extremely grateful to our illustrious group of contributors for generously sharing their time, research, clinical knowledge, and wealth of experience. This book would not have been possible without their generous support. Our book blends the most effective collaboration with the members of Springer Nature. The exceptional support of fabulous Development Editor Michael D. Sova and the most talented Editor Kristopher Spring was instrumental in seeing this book get off board from a mere concept to reality. The editors are truly obliged to their families for their love and constant support.

Campinas, Brazil — Sandro C. Esteves, MD, PhD
Hong Kong, China — Chak-Lam Cho, MD, FRCS-Ed (Urol)
Doha, Qatar — Ahmad Majzoub, MD, MBChB, CABU
Cleveland, OH, USA — Ashok Agarwal, PhD, HCLD (ABB)

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About the Editors

Sandro C. Esteves is medical and scientific director of ANDROFERT (Andrology and Human Reproduction Clinic), a referral Fertility Center for Male Reproduction in Brazil.

Dr. Sandro earned his MD in 1990 from the University of Campinas (UNICAMP), Brazil, where he did residency training in general surgery and urology. He completed his training in the United States (1995–1996) as a research fellow at the Cleveland Clinic's Center for Reproductive Medicine. He was awarded a master's degree in Surgery in 1998, at UNICAMP, and a PhD in Medicine (Urology) in 2001, at the Federal University of São Paulo (UNIFESP). He is a board-certified urologist by the Brazilian Society of Urology and a certified ART center director and IVF consultant by the Brazilian Society of Assisted Reproduction.

He is a collaborating professor in the Department of Surgery (Division of Urology) at the University of Campinas (Brazil) and honorary professor of Reproductive Endocrinology at the Faculty of Health, Aarhus University, Denmark. He is also research collaborator at both the Cleveland Clinic's Center of Reproductive Medicine (USA) and Genetic Unit, Department of Biology, Universidad Autónoma de Madrid (Spain), and holds an honorary title of clinical tutor in Urology at the University of Edinburgh (UK). His research/clinical interests include male infertility, reproductive endocrinology, assisted reproductive technology, cleanroom technology, and quality management.

Dr. Esteves has published over 200 scientific papers in peer-reviewed journals, authored over 70 book chapters, and presented over 150 papers at both national and international scientific meetings. His current Hirsch index (h-index) is 44, while his citation count is over 6,000 (Google Scholar). He is faculty member of F1000Prime in the area of reproductive endocrinology and infertility, member of the Society for Translational Medicine's Male Infertility Cooperative Group, and co-founder of the Poseidon Group (www.grouppose-idon.com).

He has served as an editor of several textbooks related to male infertility, reproductive medicine, and assisted reproductive technology. He is also the guest editor of many special issues in scientific journals on topics related to

reproductive medicine. Moreover, he is currently associate editor of the *International Brazilian Journal of Urology* and *Frontiers in Endocrinology* (Reproduction) and serves the Editorial Board of *Andrology*, *International Urology and Nephrology*, *Asian Journal of Andrology*, *Translational Andrology and Urology*, *Journal of Evidence-Based Women's Health Society*, and *MEDICALEXPRESS*.

Dr. Esteves has been invited as guest speaker in many international meetings in over 35 countries. He is the recipient of the "Alumni of the Year" Award from the Cleveland Clinic Center for Reproductive Medicine and consecutive Star Awards from the American Society for Reproductive Medicine.

Chak-Lam Cho, FRCSEd (Urol), FHKAM (Surgery) holds the position of consultant in Urology at the Department of Surgery of Union Hospital in Hong Kong since 2017.

He earned his medical degree from the Chinese University of Hong Kong in 2004 and obtained the fellowship from the Royal College of Surgeons of Edinburgh in 2012. He completed his training in male reproductive medicine at the American Center for Reproductive Medicine (Cleveland Clinic) and the Center for Male Reproductive Medicine and Microsurgery (Weill Cornell University, New York) in 2015.

He is currently the honorary clinical assistant professor at the University of Hong Kong and the Chinese University of Hong Kong. He has published over 50 scientific papers in peer-reviewed journals. His research and clinical interests include male infertility, endourological interventions for prostate and urolithiasis, and genitourethral reconstructive surgery. He is currently associate editor of *Andrologia* and serves the review editor of *Frontiers in Endocrinology* (Reproduction). He is the faculty member of the Asian Urological Surgery Training and Education Group (AUSTEG) and member of the Society for Translational Medicine's Male Infertility Cooperative Group.

Ahmad Majzoub is a specialist in the fields of Andrology and Male Infertility, who is currently working as a consultant at the Department of Urology and program director of Andrology and Male Infertility Fellowship at Hamad Medical Corporation, Doha, Qatar. He is also affiliated to Weill Cornell Medicine College, Qatar, holding a position of assistant professor. He obtained his MBChB from Beirut Arab University in 2005 and proceeded to complete his residency training in Urology in Qatar to become an Arab board-

certified urologist. He underwent a specialized 2-year training in Andrology and Male Infertility at the Hamad Medical Corporation and did a second Clinical and Research Fellowship in Andrology at the world famous Glickman Urological and Kidney Institute and the American Center for Reproductive Medicine at Cleveland Clinic Foundation, Cleveland, United States. He is also a fellow of the European Committee of Sexual Medicine.

Dr. Majzoub provides services in general urology, andrology, and male infertility, offering endoscopic, microsurgical, reconstructive, and prosthetic surgeries for his patients. His interests extend toward the management of chronic pelvic pain syndrome in men and has opened the first dedicated pelvic pain clinic in the region.

Dr. Majzoub has done extensive work in the field of medical research and has been very active with over 150 research publications at peer-reviewed journals and several book chapters mainly focusing on andrology and men's health.

He is a reviewer at several high-impact medical journals and is an active member of the American Urological Association, the American Society for Reproductive Medicine, the European and International Societies of Sexual Medicine, and the Arab Association of Urology. He holds editorial positions at a number of scientific journals and has had several speaker participations at the national and international conferences. He has served as an editor of two special issues in scientific journals on topics related to male infertility and has coedited a book entitled *The Complete Guide to Male Fertility Preservation* published by Springer International Publishing, 2018. He recently founded the male virtual clinic, an online platform dedicated for the male patient education regarding his sexual and reproductive health.

For more information, visit ResearchGate: https://www.researchgate.net/profile/Ahmad_Majzoub.

Ashok Agarwal, PhD, HCLD (ABB), ELD (ACE) is the head of Andrology Center and director of Research at the American Center for Reproductive Medicine, since 1993, of the Cleveland Clinic Foundation, where he is a professor of Surgery (Urology) at the Lerner College of Medicine of Case Western Reserve University. He was trained in male infertility and andrology at the Brigham and Women's Hospital and Harvard Medical School and later worked as an assistant professor of Urology at Harvard from 1988 to 1992. Furthermore, he has over 26 years of experience in directing busy male infertility diagnostic facilities and fertility preservation services. He is very well published with over 700 scientific papers and reviews in peer-reviewed scientific journals and is ranked in Scopus as the #1 author in the world in the fields of male infertility/andrology and human-assisted reproduction, based on the number of peer-reviewed publications, citation scores,

and h-index. He is currently an editor of 37 medical textbooks/manuals related to male infertility, ART, fertility preservation, DNA damage, and antioxidants and active in basic and clinical research. His laboratory has trained over 1,000 scientists, clinicians, and graduate and undergraduate students from the United States and more than 55 countries. His current research interests include proteomics of male infertility, molecular markers of oxidative stress, and DNA integrity in the pathophysiology of male reproduction.

Contributors

Ashok Agarwal, PhD, HCLD American Center for Reproductive Medicine, Department of Urology, Cleveland Clinic, Cleveland, OH, USA

Mohannad Alharbi, MD Division of Urology, Department of Surgery, McGill University, Montreal, QC, Canada

Department of Urology, College of Medicine, Qassim University, Qassim, Saudi Arabia

Jehan AlRayahi, MD, ABDR Diagnostic Imaging, Sidra Medicine, Doha, Qatar

Muhannad M. Alsyouf, MD Department of Urology, Loma Linda University, Loma Linda, CA, USA

Ali Amin, MD Department of Pathology and Laboratory Medicine, Rhode Island and the Miriam Hospitals/Brown University, Providence, RI, USA

Mohamed Arafa, MD Department of Andrology, Cairo University, Giza, Egypt

Department of Urology, Hamad Medical Corporation, Doha, Qatar

Ricardo Pimenta Bertolla, PhD Department of Surgery, Sao Paulo Federal University, São Paulo, SP, Brazil

Federica Bianchi, MD Department of Pediatric Surgery, Woman & Child Hospital, Department of Surgery, Dentistry, Paediatrics and Gynecology, Division of Pediatric Surgery, University of Verona, Italy, Verona, Italy

João Arthur Brunhara, MD Androscience – Science and Innovation Center and High-Complexity Clinical and Research Andrology Laboratory, Sao Paulo, Brazil

Men's Health Study Group, Institute for Advanced Studies, Division of Urology, University of Sao Paulo, Sao Paulo, Brazil

Darren J. Bryk, MD Cleveland Clinic Foundation, Glickman Urological and Kidney Institute, Cleveland, OH, USA

Gian Maria Busetto, MD, PhD Department of Urology, Sapienza Rome University, Policlinico Umberto I, Rome, Italy

Francesco Saverio Camoglio, PhD Department of Pediatric Surgery, Woman & Child Hospital, Department of Surgery, Dentistry, Paediatrics and Gynecology, Division of Pediatric Surgery, University of Verona, Italy, Verona, Italy

João Paulo Cardoso, MD Department of Urology, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, Sao Paulo, SP, Brazil

Juan D. Cedeno, MD Department of Urology, Mount Sinai Medical Center, Miami Beach, FL, USA

Peter T. K. Chan, MD, CM, MSc, FRCS(C), FACS Department of Urology, McGill University Health Centre, Montreal, QC, Canada

Peter Ka-Fung Chiu, MBChB, FRCSEd(Urol), FHKAM(Surg) Division of Urology, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

Chak-Lam Cho, FRCSEd (Urol), FHKAM (Surgery) S.H. Ho Urology Centre, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China

Matthew S. Christman, MD Department of Urology, Naval Medical Center San Diego, San Diego, CA, USA

Eric Chung, MBBS, FRACS AndroUrology Centre for Sexual, Urinary and Reproductive Excellence, Brisbane, QLD, Australia

Marcello Antonio Signorelli Cocuzza, PhD, MD Department of Urology, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil

R. Matthew Coward, MD, FACS Department of Urology, University of North Carolina School of Medicine, Chapel Hill, NC, USA

Ali A. Dabaja, MD Vattikuti Urology Institute, Henry Ford Hospital, Detroit, MI, USA

Rodrigo Uliano Moser Da Silva, MD Department of Urology, Hospital de Clínicas De Porto Alegre, Porto Alegre, Rio Grande Do Sul, Brazil

Ettore de Berardinis, MD Department of Urology, Sapienza Rome University, Policlinico Umberto I, Rome, Italy

Renata Cristina de Carvalho Department of Surgery, Division of Urology, Sao Paulo Federal University, Sao Paulo, SP, Brazil

Francesco Del Giudice, MD Department of Urology, Sapienza Rome University, Policlinico Umberto I, Rome, Italy

Rosana Maria dos Reis, PhD, MD Department of Gynecology and Obstetrics, Ribeirao Preto Medical School of University of Sao Paulo (USP), Ribeirao Preto, SP, Brazil

Alexander D. Doudt, DO Department of Urology, Naval Medical Center San Diego, San Diego, CA, USA

Vanessa L. Dudley, BA Department of Male Reproductive Medicine, Weill Cornell Medicine, New York Presbyterian Hospital, New York, NY, USA

Haitham Elbardisi, MD Department of Urology, Hamad Medical Corporation, Doha, Qatar

Sandro C. Esteves, MD, PhD ANDROFERT, Andrology & Human Reproduction Clinic, Campinas, SP, Brazil

Mohamed H. Etafy, MD Al-Azhar University Hospital, Assiut, Egypt

Department of Urology, PUR Clinic, South Lake Hospital, Clermont, FL, USA

Renato Fraietta, PhD Department of Surgery, Division of Urology, Sao Paulo Federal University, Sao Paulo, SP, Brazil

Joseph Scott Gabrielsen, MD, PhD Division of Male Reproductive Medicine and Surgery, Scott Department of Urology, Baylor College of Medicine, Houston, TX, USA

Maria Garcia-Palacios, MD, PhD Pediatric Surgery & Urology, University Hospital of Santiago, Santiago, Galicia, Spain

Ramy Abou Ghayda, MD Department of Urology, Brigham and Women's Hospital, Boston, MA, USA

Marc Goldstein, MD Department of Urology, Weill Cornell Medicine, New York, NY, USA

Angelica Anna Chiara Grasso Department of Surgery, Division of Urology, Ospedale Beata Vergine-Ente Ospedaliero Cantonale, Mendrisio, Ticino, Switzerland

Marco Grasso, MD Department of Urology, ASST MONZA, Ospedale San Gerardo, Monza, Italy

David Guo, MD Department of Urology, Rhode Island Hospital/Brown University, Providence, RI, USA

Jorge Hallak, MD, PhD Androscience – Science and Innovation Center and High-Complexity Clinical and Research Andrology Laboratory, Sao Paulo, Brazil

Men's Health Study Group, Institute for Advanced Studies, Division of Urology, Reproductive Toxicology Unit, Department of Pathology, University of São Paulo, Sao Paulo, Brazil

Taha Abo-Almagd Abdel-Meguid Hamoda, MBBS, MSc, MD Department of Urology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

Department of Urology, Faculty of Medicine, Minia University, El-Minia, Egypt

Russell P. Hayden, MD Department of Urology, Weill Cornell Medicine, New York, NY, USA

Kathleen Hwang, MD Department of Urology, Rhode Island Hospital/ Brown University, Providence, RI, USA

Hussein Kandil, MBBCh, FACS Department of Urology and Andrology, Fakih IVF Fertility Center, Abu Dhabi, United Arab Emirates

Martin Kathrins, MD Department of Urology, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, USA

Parviz Keikhosrow Kavoussi, MD, FACS Department of Reproductive Urology, Austin Fertility & Reproductive Medicine/Westlake IVF, Austin, TX, USA

Edmund Y. Ko Department of Urology, Loma Linda University, Loma Linda, CA, USA

Jason R. Kovac, MD, PhD, FACS, FRCSC Men's Health Center, Indianapolis, IN, USA

Sarah C. Krzastek, MD Department of Urology, University of Virginia, Charlottesville, VA, USA

Rajeev Kumar, MD, MCh Department of Urology, All India Institute of Medical Sciences, New Delhi, Delhi, India

Caterina Lania, MD Department of Urology, IRCCS Ospedale San Raffaele, Milan, Italy

Larry I. Lipshultz, MD Scott Department of Urology, Baylor College of Medicine, Houston, TX, USA

Daniel Lybbert, MD Department of Surgery, Division of Urology, Southern Illinois University, Springfield, IL, USA

Ahmad Majzoub, MD Department of Urology, Hamad Medical Corporation, Weill Cornell Medicine Qatar, Doha, Qatar

Joel L. Marmar, MD Cooper University Hospital, Camden, NJ, USA

Surgery/Urology, Cooper Medical School of Rowan University, Camden, NJ, USA

Daniel J. Mazur, MD Scott Department of Urology, Baylor College of Medicine, Houston, TX, USA

J. Abram McBride, MD Scott Department of Urology, Baylor College of Medicine, Houston, TX, USA

Benjamin J. McCormick, MD Department of Urology, University of North Carolina School of Medicine, Chapel Hill, NC, USA

Richard A. Mendelson, PhD Department of Graduate Psychology, Keiser University, Ft. Lauderdale, FL, USA

Roberto Mendez-Gallart, MD, PhD Pediatric Surgery & Urology, University Hospital of Santiago, Santiago de Compostela, A Coruña, Spain

Cristiana Libardi Miranda-Furtado, PhD Department of Gynecology and Obstetrics, Ribeirao Preto Medical School of University of Sao Paulo (USP), Ribeirao Preto, SP, Brazil

Walid M. Mubarak, MBBCh, ABDR, DipHCM Diagnostic Imaging – Interventional Radiology, Sidra Medicine, Doha, Qatar

Bryan Naelitz, BA Glickman Urological and Kidney Institute, Cleveland Clinic Lerner College of Medicine, Cleveland, OH, USA

Craig S. Niederberger, MD Department of Urology, University of Illinois at Chicago Hospital and Health Sciences System, Chicago, IL, USA

Samuel J. Ohlander, MD Department of Urology, University of Illinois at Chicago Hospital and Health Sciences System, Chicago, IL, USA

Fatima Kazue Okada, PhD Department of Surgery, Sao Paulo Federal University, São Paulo, SP, Brazil

Rodrigo L. Pagani, MD Department of Urology, University of Illinois at Chicago Hospital and Health Sciences System, Chicago, IL, USA

Sijo J. Parekattil, MD Personalized Urology & Robotics, The PUR Clinic & University of Central Florida, Clermont, FL, USA

Neel Parekh, MD Glickman Urologic and Kidney Institute, Cleveland Clinic, Cleveland, OH, USA

Alexander W. Pastuszak, MD, PhD Department of Surgery, Division of Urology, University of Utah School of Medicine, Salt Lake City, UT, USA

Simone Patanè, MD Department of Pediatric Surgery, Woman & Child Hospital, Department of Surgery, Dentistry, Paediatrics and Gynecology, Division of Pediatric Surgery, University of Verona, Italy, Verona, Italy

Marta Peretti, MD Department of Pediatric Surgery, Woman & Child Hospital, Department of Surgery, Dentistry, Paediatrics and Gynecology, Division of Pediatric Surgery, University of Verona, Italy, Verona, Italy

Alan Scott Polackwich Jr., MD Columbia Department of Urology at Mount Sinai Medical Center in Miami Beach, Mount Sinai Medical Center, Miami Beach, FL, USA

S. V. Krishna Reddy, MS,MCh, (Urology) Department of Urology, Narayana Medical College & Hospital, Nellore, Andhra Pradesh, India

Ashley James Robinson, BSc MB ChB Diagnostic Imaging, Sidra Medicine/Weill-Cornell Medical College, Doha, Ad Dawhah, Qatar

Matheus Roque, MD, PhD Department of Reproductive Medicine, ORIGEN – Center for Reproductive Medicine, Rio de Janeiro, Brazil

Connor Rotterman, MD Men's Health Center, Indianapolis, IN, USA

Giorgio Ivan Russo Department of Surgery, Urology Section, University of Catania, Catania, Italy

Edmund Sabanegh Jr., MD, MBA Executive Administration, Cleveland Clinic, Cleveland, OH, USA

Mary K. Samplaski, MD Institute of Urology, University of Southern California, Los Angeles, CA, USA

Jay I. Sandlow, MD Department of Urology, Medical College of Wisconsin, Milwaukee, WI, USA

Viviane Paiva Santana, MSc Department of Gynecology and Obstetrics, Ribeirao Preto Medical School of University of Sao Paulo (USP), Ribeirao Preto, SP, Brazil

Manesh Kumar Panner Selvam, PhD American Center for Reproductive Medicine, Department of Urology, Cleveland Clinic, Cleveland, OH, USA

Ege Can Serefoglu, MD, FECSM Department of Urology, Bahceci Health Group, Istanbul, Turkey

Luke E. Sewall, MD Department of Interventional Radiology, Vascular and Interventional Professionals, Hinsdale, IL, USA

Adit Shah, MD Institute of Urology, University of Southern California, Los Angeles, CA, USA

Rupin Shah, M.Ch. (Urology) Department of Urology, Lilavati Hospital Research Centre, Mumbai, India

Ahammad Basha Shaik, MSc, PhD, (Statistics) Department of Community Medicine, Narayana Medical College & Hospital, Nellore, Andhra Pradesh, India

Mark Sigman, MD Department of Surgery (Urology), Brown University/ Lifespan, Providence, RI, USA

Luriel I. Smith-Harrison, MD Department of Urology, Medical College of Wisconsin, Milwaukee, WI, USA

Ryan P. Smith, MD Department of Urology, University of Virginia, Charlottesville, VA, USA

Steven Janney Smith, MD Department of Interventional Radiology, Vascular and Interventional Professionals, Hinsdale, IL, USA

Phillip K. Stokes, MD Department of Urology, Loma Linda University, Loma Linda, CA, USA

Nicholas N. Tadros, MD, MCR Department of Surgery, Division of Urology, Southern Illinois University, Springfield, IL, USA

Thiago Afonso Teixeira, MD, MSc Androscience – Science and Innovation Center and High-Complexity Clinical and Research Andrology Laboratory, Sao Paulo, Brazil

Men's Health Study Group, Institute for Advanced Studies, Division of Urology, University of Sao Paulo, Sao Paulo, Brazil

School of Medicine, Federal University of Amapa, Macapa, Brazil

Nannan Thirumavalavan, MD Urology Institute, University Hospital / Case Western Reserve University, Cleveland, OH, USA

Danielle Velez, MD Department of Urology, Brown Urology/Rhode Island Hospital, Providence, RI, USA

Sarah C. Vij, MD Cleveland Clinic Foundation, Glickman Urological and Kidney Institute, Cleveland, OH, USA

Guilherme J. A. Wood, MD Department of Urology, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil

Siddharth Yadav, MBBS, MS (Gen.Surg), MCh (Urol) Department of Urology and Renal Transplant, Vardhaman Mahavir Medical College & Safdarjung Hospital, New Delhi, Delhi, India

Grace Yaguchi, MD Vattikuti Urology Institute, Henry Ford Hospital, Detroit, MI, USA

Nicola Zampieri, MD, PhD Department of Pediatric Surgery, Woman & Child Hospital, Department of Surgery, Dentistry, Paediatrics and Gynecology, Division of Pediatric Surgery, University of Verona, Italy, Verona, Italy

Armand Zini, MD Division of Urology, Department of Surgery, McGill University, Montreal, QC, Canada

Daniel Suslik Zylbersztejn, MD, PhD Fleury Group, São Paulo, SP, Brazil

Part I

Varicocele Origin and Pathophysiology

1 The Evolution and Refinement of Varicocele Treatment: A Historical Perspective

Joel L. Marmar

Key Points

- Varicoceles were recognized since antiquity because they were visible, palpable and painful.
- During the "Pain Era" (first to nineteenth centuries), varicocele surgery was limited to the scrotum. Although these procedures often provided pain relief, the true pathophysiology was poorly understood.
- The development of the "microscope" in 1677 enabled scientists to recognize that "sperm" were present in the semen, and that men could contribute to barren marriages.
- The "Fertility Era" did not begin until 1957, when the first patient had varicocele surgery for the treatment of male infertility. Thereafter, the number of varicocele surgeries increased dramatically.
- New techniques related to "hernia surgery," "microsurgery" and "venography with embolization" led to advancements for "varicocele repair." Furthermore, "venography" documented retrograde blood flow into the internal spermatic veins as the cause of varicoceles.
- New laboratory studies revealed the "Pathophysiology of Varicoceles" to include reactive oxygen species (ROS) in the semen, DNA damage to sperm, seminal protamine changes and low serum testosterone. Furthermore, several studies revealed that these findings could be reversed by "Varicocele Surgery."

Introduction

Varicoceles have had a long and fascinating history, and this chapter will present the readers with comprehensive information regarding the diagnosis, pathophysiology and treatment of these lesions.

Historically, varicoceles were recognized since antiquity and surgical repairs were initiated in the first century AD. The original treatments were performed for pain management, and these years were known as the "The Pain Era." When venography was popularized, dyes were injected into the internal spermatic veins, and retrograde blood flow was identified as the basic pathologic phenomenon.

J. L. Marmar (✉)
Cooper University Hospital, Camden, NJ, USA

Surgery/Urology, Cooper Medical School of Rowan University, Camden, NJ, USA

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In 1952, the first repair of a varicocele was reported for the treatment of male infertility. Thereafter, the number of varicocele surgeries increased greatly and these years became known as "The Fertility Era." As a result of the increased interest in the treatment of varicoceles, a variety of surgical adaptations were introduced that included microsurgery, laparoscopy and robotic surgery. In addition, venography with embolization and sclerosis were used to treat these lesions.

In addition, animal models were developed to clarify the pathophysiology of varicoceles, and extensive human studies among men with varicoceles documented abnormal semen parameters, evidence of sperm DNA damage, factors related to oxidative stress, changes in heat shock proteins and lowered serum testosterone. Recently, sperm from men with varicoceles were used for in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) and some studies suggest improved delivered babies born with these procedures after varicocelectomy.

Therefore, Chap. 1 will provide the introductory remarks and offer a historical perspective for the many topics currently associated with varicoceles. Then, the other chapters of this book will provide both comprehensive and current information related to varicoceles.

Early History of Varicoceles

Clinicians, researchers and patients have been interested in varicoceles for many centuries, and several comprehensive review articles have been written on this subject [1–4]. These articles suggest that the recognition of varicoceles may actually date back to antiquity because some Greek statuary and Egyptian art depicted men with scrotal swellings. Although some enlargements may have been hernias or hydroceles, the authors assumed that some of these enlargements were varicoceles.

The earliest surgery performed on a varicocele was recorded in the first century AD, and it was attributed to the Roman physician Celsus. This scholar wrote in Latin about many medical/surgical topics of his era in a document known as *De Medicina*. Celsus referred to the suspected varicoceles as a "Circoceles," and his surgeries were accomplished by a scrotal approach.

At that time, the lesions were associated with pain, and the surgery required anesthesia. Therefore, Celsus dampened a sponge with opium and other agents, and he placed it below the nostrils. This technique was well established during Roman times because it was commonly used for anesthesia during crucifixions.

The original varicocele surgery was limited to the scrotum. After the intrascrotal veins were exposed, they were ligated. The testicular arteries and vas deferens were avoided, but the role of these structures was not well understood. The surgery was completed after the external veins on the surface of the scrotum were cauterized by direct puncture with a hot rod.

Over the centuries, many scholars have studied the classic writings of Celsus by reading the original Latin text, but it was particularly fitting for Robert Hotchkiss, MD, of New York University, to reference the work of Celsus in his book "Male Infertility," which was published in 1955. Although the lives of Celsus and Hotchkiss were separated by 2000 years, there was a significant connection between them because in his time, Dr. Hotchkiss was recognized as the father of modern andrology.

Furthermore, Dr. Hotchkiss teamed with John MacLeod, PhD of Cornell, and together they reported extensively on the fundamentals of the semen analysis, and they stimulated many of their students to pursue studies related to varicoceles [5]. However, perhaps the most important idea that was proposed by Drs. Hotchkiss and MacLeod was their suggestion that the study of "male infertility" should be considered as a subspecialty within the field of Urology. Since this suggestion has become a reality, it will be rewarding for the readers of this book to note that the study of varicoceles was a cornerstone for the development of the field of male infertility.

The Pain Era

Even in ancient times, there seemed to be a need to correct varicoceles because these lesions were

visible, palpable and painful. Although there were no tools to properly study the anatomy and pathophysiology of varicoceles in ancient times, the crude surgeries were a testimony to curiosity and ingenuity of these early clinicians, and this period was known as the "Pain Era."

The "Pain Era" lasted from the first to the nineteenth centuries. The lesions were identifiable, but the anatomy and the pathophysiology were not clearly understood. Nevertheless, Rothman [6] compiled a collection of techniques that were used to treat varicoceles during the "Pain Era." These included acupuncture, direct puncture of veins and placement of nonabsorbable thread into the veins which were removed when the veins dried-up, etc. In addition, the use of "bloodletting" was commonly practiced because the early clinicians believed that varicoceles contained "melancholic blood."

Since the standard means of transportation in the early days was by horseback, it seems reasonable to assume that this activity may have provoked scrotal pain for many men with varicoceles. In one such case, a famous surgeon of his day, Sir Astley Pastor Cooper, wrote about his new technique for varicocele surgery. He referred to a varicocele as "Orchidoptosis," and he performed a unique procedure that included reduction of the scrotal sac as the treatment. At one point, Cooper spoke about a patient on whom he performed varicocele surgery, and he bragged that, after his procedure, this patient was able to ride 50 miles on horseback without pain [7].

In time, new ideas concerning all areas of science and medicine were being reported, and some of these ideas were directly applied to the study of varicoceles. For example, the microscope was introduced by Antonie van Leeuwenhoek in Delft in 1677 [8], and he was the first to identify sperm in the semen. He called the sperm "animalcules" because they demonstrated movement by the action of their tail. However, it was not until early in the nineteenth century before a British physician named Thomas Blizzard Curling used the microscope in clinical practice and proposed several new ideas regarding varicoceles. Mr. Curling specialized in the treatment of testicular disorders, and he was among the founders of the new specialty called "endocrinology." He was fascinated by microscopy and he used this instrument to study men with sterility. At that time, it was still unclear whether men could be responsible for some barren marriages, but Mr. Curling resolved the issue with his semen studies.

In addition, Mr. Curling studied the semen of men with varicoceles, and he created hand drawings of different sperm shapes which may represent the first classification of sperm morphology. Also, he had other creative ideas about the examination and treatment of varicoceles. Mr. Curling was the first to use the term, "varicocele," and he recommended that the patients be examined in the upright position, because this simple maneuver fully expanded the scrotal veins. Still further, he was among the first to suggest that these enlarged veins may transmit excess heat to affect the testes.

Mr. Curling introduced a new surgical procedure whereby he removed a major portion of the scrotal sac to achieve better support for the testes. In addition, he utilized a scrotal supporter, and he proposed "scrotal fanning" to cool the testes. Although these approaches may seem crude by current day standards, these basic ideas started other investigators to think about the pathophysiology and management of varicoceles. Furthermore, Mr. Curling's interest in semen microscopy linked varicoceles to fertility-related problems, and some scholars have suggested that Mr. Curling's work actually began the "Fertility Era," which will be detailed later in this chapter. Although Mr. Curling was a surgeon in England, an extensive report about his work was written in French [9], which also confirms an international interest in varicoceles.

The Influence of Hernia and Abdominal Surgeries on the Correction of Varicoceles

In several articles written about varicoceles, the authors described new ideas for hernia repairs that were adaptable to varicocele surgery. Furthermore, it was interesting that these ideas

originated from diverse locations around the world. For example, Bassini [10] performed a procedure called "The Radical Operation of the Inguinal Hernia" in Italy, and he utilized an inguinal approach. Shortly thereafter, in Germany, Narath [11] used the Bassini approach for the correction of varicoceles when he moved the operative site from the scrotum to the inguinal area.

In Argentina, Ivanissevich [12] studied cadavers and identified the vessels of the spermatic cord. He introduced a supra inguinal approach for the repair of varicoceles because there were fewer venous trunks to ligate at this location, and he observed that retrograde blood flow occurred in men with varicoceles. Furthermore, at this level, it was easier to avoid injury to the testicular artery, and in 1960, he reported his experience with over 4000 cases using this approach.

Soon thereafter in Guatemala, Palomo [13] introduced the "Radical Cure of a Varicocele by a New Technique." After studying the anatomy of the spermatic cord as it exited just above the internal inguinal ring, he concluded that there were three arteries within the spermatic cord at this location, and there were fewer venous branches at this level of the inguinal canal. He reasoned that the varicocele repairs were effective, as long as he ligated only two arteries and all of the veins. If one artery remained open, then there would be enough blood flow to maintain testicular circulation.

Thus, the diagnosis of varicoceles was indeed a worldwide problem, and thoughtful innovations for repair were proposed in many countries.

The Fertility Era

Clinical utilization of the semen analysis was just beginning in the 1940s, and some investigators started to use the hemocytometer for sperm counting. Curiously, two of these investigators worked as a team in New York City and both of these men had an interest in the diagnosis and treatment of varicoceles. They were John MacLeod, PhD, and Robert Hotchkiss, MD. At Cornell, Dr. MacLeod completed his PhD thesis entitled "The Metabolism of Human Spermatozoa." At NYU, Dr. Hotchkiss rose through the ranks and became the Director of the Department of Urology, and he published an important book entitled "Male Fertility." In addition, Drs. MacLeod and Hotchkiss published a classic manuscript entitled "Semen Analysis in 1500 Cases of Sterile Marriage" [14].

Although the most common reason for varicocele repair in the early days was for the relief of pain, the role of varicocele surgery was about to change dramatically. In 1952, Tulloch [15] was the first clinician to repair a varicocele for the treatment of infertility. The patient presented with azoospermia, but after the procedure sperm returned to the ejaculate and the patient achieved a pregnancy.

Tulloch utilized the Robb procedure [16], which accessed the spermatic veins about 5 cm above the internal inguinal ring. At the end of one of his surgical procedures, Robb observed evidence of the retrograde venous flow associated with varicoceles after he injected dye into an enlarged vein. Based on his finding, Dr. Tulloch convinced others to utilize this type of surgery. For example, Charles Charny, MD, was based in the United States, but he traveled to the United Kingdom and observed Tulloch perform varicocele surgery on infertile males. When Dr. Charny returned home, he was the first to perform this procedure in the USA. In addition, Dr. Charny was a pioneer in the study of testis biopsies and he reported the differences in testis histology among infertile men with varicoceles [17] which may have represented an early study related to the pathophysiology of varicoceles.

In time, the incidence of varicoceles has proven to be substantially greater among men with infertility. For example, Clavijo et al. [18] recently updated the incidence of varicoceles among three separate study populations. For men with normal fertility, the incidence of varicoceles was 4.4–22.6%. Among those with primary infertility, the incidence of varicoceles was 45%, but among men with secondary infertility, the incidence of varicoceles was about 80%. Although these findings suggested that not all men with varicoceles were infertile, it seemed reasonable to

assume that the presence of varicoceles was commonly associated with infertility. Thus, from this point, varicoceles were forever associated with infertility, and during the "Fertility Era," there has been a steady expansion of studies related to the pathophysiology and treatment of these lesions.

In fact, several basic studies related to varicoceles were reported by several students of Hotchkiss and MacLeod. These students completed their Urology training, and they entered into the relatively new specialty of "Male Infertility." Drs. Dubin and Amelar entered into a practice together and became recognized for their clinical work and their publications related to varicoceles. In one manuscript, they proposed a grading system for the classification of varicoceles [19] that is still used today. They suggested that these men should be examined in the upright position. A Grade III varicocele was both visible and palpable, a Grade II varicocele was only palpable and a Grade I lesion was palpable only during a Valsalva maneuver. In 1975, Dubin and Amelar applied their diagnostic grading system for varicoceles to the semen analyses of 504 men with varicoceles who had not produced a pregnancy for at least 1 year [20].

In addition, Brown et al. [21] presented semen data and they confirmed that men with varicoceles had lower sperm counts than men without these lesions, but the true basis for the underlying cause of the infertility remained unclear.

Zorgniotti and MacLeod [22] proposed the idea that the varicoceles produced higher temperatures within the scrotum and testis that led to infertility, and Zorgnoitti and Sealfon [23] developed a scrotal cooling device to treat the infertility associated with these lesions. Thus, the "Fertility Era" had a strong beginning.

The Changing Role of Interventional Radiology, Thermography and Scrotal Ultrasound

The diagnostic evaluations of a varicocele at the beginning of the "Fertility Era" included a physical examination of the scrotum and semen analyses. However, new tests were introduced that clearly identified the anatomical differences that were associated with varicoceles.

In 1966, Ahlberg et al. [24] utilized selective venography to demonstrate and confirm retrograde blood flow from the internal spermatic veins into the pampiniform plexus. Although these studies were initially diagnostic, it became apparent that they may be used for correction of varicoceles, as well. Subsequently, Comhaire and Kunnen [25] suggested that venography may prove useful for venous occlusion, and Lima et al. [26] performed sclerosis of the internal spermatic veins by repeated delivery of hypertonic glucose in small doses. As an alternative, Kunnen [27] utilized the tissue adhesive (2-isobutyl-cyanoacrylate) bucrylate to harden the lumen of the internal spermatic veins which eliminated the retrograde blood flow.

In clinical practice, Comhaire and Kunnen [28] used this material on 97 infertile men with varicoceles. Although their overall pregnancy rate was 50.5%, this procedure was discontinued when Fernandez Aparicio et al. [29] experienced extensive hardening of the abdominal venous system that required emergency open surgery for removal of the hard clot. Nevertheless, in some cases of surgical failures, percutaneous venography and embolization were still used to eliminate the reflux. As an alternative, Morag et al. [30] used occlusive steel coils to eliminate the reflux, whereas Walsh and White [31] used balloons.

Although Zorgniotti and MacLeod [22] had already reported data to confirm scrotal warming in men with clinical varicoceles, Comhaire et al. [32] introduced contact thermography to distinguish between clinical and subclinical lesions. In practice, Comhaire [33] used a heat-sensitive thermal screen to evaluate varicoceles before and after surgery or embolization (Fig. 1.1 and Table 1.1). At that time, the finding of scrotal warming was considered the leading phenomenon to explain the pathophysiology of varicoceles, and several additional therapeutic devices were utilized in clinical practice to produce a cooling effect. Zorgniotti et al. [34] published reports to document the pregnancy rates after the use of these devices, and Osman et al.

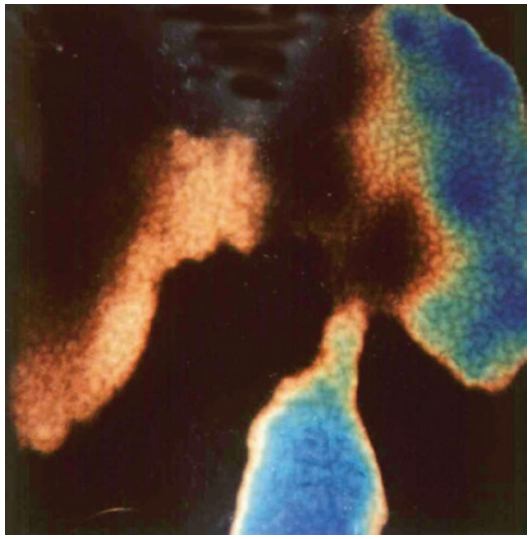

Fig. 1.1 A thermal contact strip was positioned against the scrotum while a patient with a left varicocele stood upright [32]. The color change demonstrated increased heat within the left hemi scrotum

Table 1.1 The use of a thermal strip to document increased heat from a left varicocele

1. The patient stood upright for the examination
2. The thermal strip was placed in contact with the scrotum
3. The patient performed a Valsalva maneuver
4. If a significant varicocele is present, a blue color change is noted on the strip to indicate heat (the base of the penis was blue, as well, indicating heat)
5. This patient had a left varicocele
6. This device documented one aspect of the pathophysiology of varicoceles.

Please, note Fig. 1.1

[35] introduced the application of scrotal cooling patches as part of a randomized controlled trial among men with varicoceles and infertility which continued to support the negative role of a heat factor in association with a varicocele.

As part of the diagnostic workup of varicoceles, scrotal ultrasound was introduced to evaluate the blood flow associated with these lesions. Specifically, Rifkin et al. [36] used a Doppler probe to enable the examiner and the patient to hear the sound of the arterial beat and the retrograde blood flow. While the patient stood upright, the probe was positioned over the inguinal area to localize beating testicular artery of the spermatic cord. When the patient was asked to perform a Valsalva maneuver, the sound of the retrograde flow was audible which confirmed the presence of a varicocele. At that time, both the patient and the doctor were aware of the sound, and the reflux was very convincing to the patient that he had retrograde blood flow. If no varicocele was present, there was no other sound except the arterial beat.

As the ultrasonic technology improved, more complex information was learned about these lesions. For example, Bagheri et al. [37] introduced a new technique that utilized color Doppler ultrasound (CDUS) in a way that was predictive of the semen findings and the fertility potential of patients with varicoceles. First, the examiner needed a timing measurement of the venous reflux. If the time was more than 1000 ms, it was considered pathologic [38]. Furthermore, specific reflux patterns were identified with the ultrasonic device, and these patterns correlated well with the sperm count and motility. The specific terms to classify the reflux patterns were retrograde, augmentation, enhancement and stasis.

These patterns were retained on the screen and they could be stored as a permanent record. Since these types of studies are noninvasive, they have an advantage of being easily repeated following surgery and the ultrasonic patterns may be correlated with the results of the semen analyses.

Utilization of Animal Models to Study the Pathophysiology of Varicoceles

During the "Fertility Era," it became apparent that men with varicoceles manifested clinical diversity. Some men were fertile and had children, whereas others with varicoceles remained infertile. Although the source of the pathophysiology of varicoceles remained elusive, many lessons were learned in the laboratories from the studies on animal models with varicoceles. Several laboratories created these models by partial occlusions of the renal veins and the internal spermatic veins. For example, in 1979, Al-Juburi et al. [39] presented data from one of the early animal studies. They created a partial obstruction to the left

renal vein medial to the entrance of the left internal spermatic vein in dogs. The semen volume was not affected in these models, but the semen analysis revealed that the sperm count, motility, percentage of viable sperm and percentage of oval sperm decreased significantly.

In 1981, Saypol et al. [40] studied rats and dogs. In the rats, the left renal veins were partly occluded medial to the entrance of the spermatic vein. In seven dogs, the internal valves of the left spermatic veins were destroyed completely. Compared to controls, the number of late spermatids and spermatozoa were significantly reduced in the experimental animals, and these findings confirmed that the varicocele affected sperm quality.

In the same laboratory, Hurt et al. [41] created long-term varicoceles in rats, and the lesions were corrected after 100 days. The animals were re-evaluated 60 days after the repair by micropuncture samples from the epididymis. Although the sperm motility declined after the creation of the varicocele, the motility returned to normal following the repair. Still further in the same laboratory, Turner et al. [42] noted a decrease in the Leydig cell population and in 2001 they reported a reduction of the intratesticular testosterone following the establishment of an experimental varicocele. This finding alerted clinicians to evaluate serum testosterone in human patients with varicoceles.

Based on these animal model studies, Turner [43] presented yet another provocative question. Why did a unilateral left varicocele cause bilateral effects? In the years ahead, this question was investigated in humans, and the findings shed new light on the pathophysiology of varicoceles.

New Laboratory Findings Related to the Pathophysiology of Varicoceles in Humans

Recently, several investigators presented new and dramatic molecular findings to explain the pathophysiology of varicoceles. For example, Krzysciak and Kozka [44] reported that the retrograde blood flow within the walls of varicose veins increased the pressure that released products associated with oxidative stress. Soon thereafter, Santoro and Romeo [45] were the first to specifically document that increased reactive oxygen species (ROS) and nitrous oxide (N_2O) were identified bilaterally in cases of a unilateral varicocele. In addition, Mostafa et al. [46] measured the ROS levels in men with varicoceles, and they determined that grade of the varicocele correlated with the ROS levels.

In the past, several investigators advocated the empirical use of antioxidants as part of the treatment of varicoceles, but Showell et al. [47] of the Cochrane Collaborative were hesitant to recommend antioxidants because of the lack of convincing data from prospective randomized trials. Recently, Garg and Kumar [48] presented favorable new data from randomized trials that suggested that antioxidants may be beneficial for the treatment of varicoceles. Overall the Cochrane Collaborative was originally hesitant to recommend specific treatment of varicoceles for infertile men, but they changed their position on varicocele surgery. In their most recent review, they stated that there is now sufficient evidence based on data from randomized prospective trials suggesting that treatment of a varicocele may improve a couple's chance of pregnancy [49].

In addition, other supportive laboratory data have become available from studies on infertile men with varicoceles, and these results introduced new types of testing for this group. For example, Zini et al. [50] reported an association of sperm DNA damage among men with varicoceles that was reversed following varicocele repairs. Furthermore, Deepinder et al. [51] carried out studies to measure the amount of ROS in the semen of infertile men with varicoceles with new technology. Still further in a separate investigation, Agarwal et al. [52] carried out proteomic analyses on the semen of infertile men with varicoceles. In some cases, the data documented the presence of heat shock proteins which may identify heat sensitive men with varicoceles. In still other studies by Agarwal et al. [53], varicoceles seemed to affect the Leydig cells of some men, which may produce low serum testosterone levels in some men with varicoceles.

As a result of these findings, Cho et al. [54] suggested that additional laboratory studies may be considered for the basic workup of patients with varicoceles, and expanded discussions of these topics will be presented in other chapters of this book.

The Impact of Revised Standards for Semen Analyses

In order to create uniform protocols for the workup of varicoceles and infertility, clinical guidelines were developed by three societies. They included a joint report from the American Society of Reproductive Medicine and the American Urologic Association [55] and a separate report by Dohle et al. of the European Urologic Association [56]. However, the semen data in these guidelines were based on the World Health Organization standards prior to 2010. Although the goal of these organizations was to create uniformity for the work-up of varicoceles, it became clear that new questions that needed to be answered. For example, the WHO manual on semen analysis [57] originally reported levels for semen abnormality that were based on the data from infertile men, However, in 2010, the WHO revised its manual. They reported findings that were recorded from over 1900 semen analyses, and these data were based on the findings from fertile men [58]. Although the baseline values for normality of semen parameters were revised downward, Kruger [59] reviewed the subject and despite these revisions, he concluded, "Based on available evidence, it is clear that there is a benefit in treating men with a palpable varicocele."

Surgical Developments—Microsurgery

Although varicocele surgery had been used widely for the treatment of male infertility, these procedures were not without notable complications such as injury to the spermatic arteries or disruption of the spermatic cord lymphatics. For example, Silber [60] reported a case in which the internal spermatic arteries were ligated during a varicocelectomy, and he recommended the use of ocular loops for the dissection of the cord structures. In a separate report, Woznitzer and Roth [61] reported that a surprising number of arteries were found in the specimens that were removed during the varicocele dissection. Therefore, these investigators suggested the use of an operating microscope, microsurgical instruments and a Doppler ultrasound probe during a varicocele repair to avoid injury to the spermatic cord arteries and the lymphatics.

Subsequent to these types of reports, Marmar et al. [62] published the first subinguinal microsurgical varicocelectomy in 1985, by using an operating microscope and microsurgical instruments to avoid complications. This team had developed considerable confidence with microsurgery by performing vasectomy reversals, and they readily adapted their techniques to varicocele repairs (Fig. 1.2 and Table 1.2).

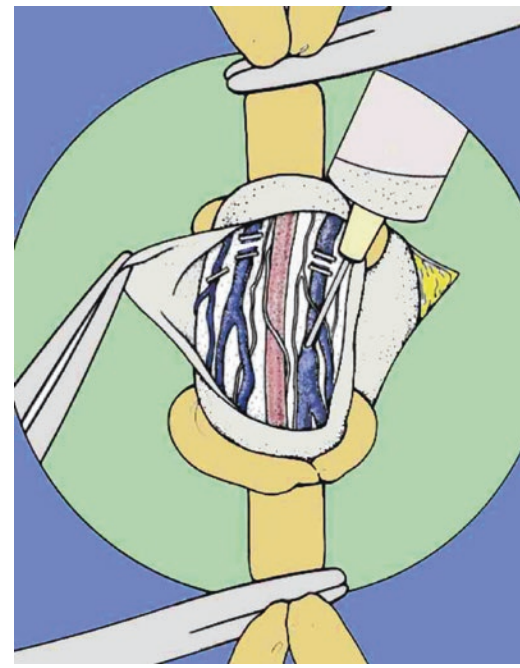

Fig. 1.2 Microsurgery of a varicocele demonstrates preservation of the lymphatics and testicular artery, occlusion and transection of varicose veins >2 mm and sclerosis of the remaining small veins (a small clip is used to seal the puncture site—not shown) [62]

Table 1.2 The sub-inguinal microsurgical varicocelectomy

1. The patient receives limited IV sedation and 2–3 cc of 1% Xylocaine in the inguinal skin
2. A 2 cm incision is completed in the area below the inguinal canal
3. The spermatic cord is identified and elevated above the skin level with a Babcock clamp
4. Penrose drains are placed behind the spermatic cord, and the structure is elevated secured above the skin level
5. The superficial veins on the fascia of the spermatic cord are dissected under magnification with micro instruments, sealed with hemoclips and the veins are cut
6. The superficial fascia is opened in two layers to expose the structures within the spermatic cord
7. The lymphatics are identified and avoided
8. If the arterial beat is weak, droplets of Papaverine are dripped onto testicular artery to augment the beat
9. Veins within the spermatic cord greater than 2 mm are micro-dissected, clipped with hemoclips and cut
10. At this point, the Penrose drains are cinched around the spermatic cord
11. A 5 cc syringe is filled with Sotrodechol and a 30G needle is attached to the tip
12. A vein is punctured with the needle tip and 0.5–2 cc of sclerosant is introduced. The fluid is observed as it flows through the venous structures
13. When the needle is removed from the vein, the pinhole opening is sealed with a single hemoclip
14. The Penrose drains are removed, the spermatic cord is replaced into its normal position and the skin is closed with a 3–0 nylon suture

Please note the drawing in Fig. 1.2

Marmar and Kim [63] summarized their data related to their microsurgical varicocelectomy experience. They reported the data on 466 patients who had 606 procedures. There were no arterial injuries, there was only one hydrocele that required correction, and the palpable recurrence rate was 0.82% based on the total number of procedures. The 1-year pregnancy rate was 35.6% based on the follow-up of 186 post varicocelectomy patients. In a separate report, Gontero et al. [64] compared the inguinal versus subinguinal approaches, and they reported visual analog pain scores (VAS) for each procedure. Although more veins were encountered with the subinguinal approach, the VAS scores were significantly higher for the inguinal approach ($p = 0.008$).

In 1992, Goldstein et al. [65] modified the microsurgical, subinguinal varicocelectomy in several ways. They performed 640 procedures on 429 men, and they delivered the testes during the procedure in order to ligate the gubernacular veins. These surgeons did not use sclerosis during their procedures, because they ligated all of the visible veins of the spermatic cord. They reported a failure rate of 0.6% for all procedures and a pregnancy rate of 43% at 6 months. However, at the same institution, Ramasamy and Schlegel [66] compared the results of microsurgical varicocelectomies with delivery of the testis (55 patients) versus cases without testis delivery (110 patients). At 1 year, the pregnancy rate with testis delivery was 40.0% versus 55.0% without delivery, but these differences were not significant. Nevertheless, there seemed to be more operative inflammatory reaction to the scrotum and a longer operating time in cases with delivery of the testis from the scrotum.

Antegrade Scrotal Sclerotherapy

This procedure represents an off-shoot of other radiographic techniques that have been used for the management of varicoceles, and these procedures have been used on both adolescents and adults. Although there have been mostly favorable results, there have been some significant complications that will require specific discussion. Furthermore, since these procedures have been used on adolescents, this topic will be discussed in detail in a separate section of this book.

Tauber and Johnsen [67] have been credited with the introduction of antegrade scrotal sclerosis for the treatment of varicoceles, and their initial series included 218 patients. Preoperatively, these individuals had scrotal pain, increasing varicocele size or low sperm density/infertility. After a local anesthetic was injected into the upper scrotal skin, a 1–2 cm incision exposed the spermatic cord. Then a 24G cannula was inserted into a straight vein of the spermatic cord in the antegrade direction, the patient was placed in the Trendelenburg position and he performed a

Valsalva. Once in position, the cannula was secured with a single suture, and about 1 cc of air followed by 3 ml of sodium morrhuate were injected during the Valsalva.

The follow-up reported no varicocele in 91% of the cases and 42% of the infertile cases conceived within 3–30 months. Although these results were favorable, significant complications have been reported in other series. For example, Goll et al. [68] reported the loss of a testicle due to an infarct after one of these procedures. In a separate study, Salerno et al. [69] documented that an anomalous anastomosis may occur between the left internal spermatic and visceral veins, and they suggested venography before sclerosis. Since Vicini et al. [70] reported a large bowel infarct following antegrade sclerosis, Tauber et al. [71] also recommended preliminary phlebography for his 5424 cases before antegrade sclerosis.

Robotic-Assisted Varicocele Surgery

As new technology was introduced, new surgical techniques were reported. Corcione et al. [72] were the first to use a robotic-assisted platform as part of a laparoscopic varicocelectomy, and Shu et al. [73] performed the first eight robotic-assisted subinguinal varicocelectomies. They compared the follow-up data to the results of eight conventional microsurgical procedures; the operating times were the same, and neither had complications. Recently, Parekattil and Gudeloglu [74] published a comprehensive review of robotic assistance for andrology, and they noted excellent results. However, they pointed out that the equipment and insurance costs for robotic surgery are substantially greater than conventional surgery. Thus it remains to be seen whether robotic surgery can compete with conventional microsurgery on a regular basis.

Debatable Topics

The utilization of assisted reproductive techniques has become commonplace in the management of male infertility, and Pathak et al. [75] reviewed the outcomes of Assisted Reproductive Techniques (ART) for infertile men with varicoceles. Some data suggest that a varicocele repair did not reduce the need for IVF, whereas Kohn et al. [76] have suggested that varicocele surgeries may improve the results of IVF/ICSI. Samplaski et al. [77] suggested that a varicocele repair may provide opportunities for pregnancies with Intra Uterine Insemination (IUI), because of sufficient improvement in the sperm count. Clearly, these issues need additional study, and there will be further discussion of this topic in the other chapters of this book.

The surgical repair of varicoceles in adolescents is another topic that has been debated. Some investigators suggest that the presence of a varicocele may be progressive [78], and most pediatric urologists suggest these lesions should be repaired to prevent future infertility, especially if there is reduction of testicular size [79]. Semen analyses have been successfully carried out to study the fertility of mature adolescents [80], but these studies have not been universally accepted by all of the families. The question that needs additional study is whether the current testing can determine which adolescents will experience infertility in adulthood?

Lastly, Sirvent et al. [81] reported that varicoceles may affect Leydig cell function. With aging, these men may demonstrate reduction of serum testosterone. Recently, Dabaja and Goldstein [82] reviewed the literature on this subject, and they suggested that varicocelectomy may improve the serum testosterone levels in these adults. Will this mean that the treatment of varicoceles may be recommended for aging males with low testosterone?

The Book

The authors are confident that the chapters within this book will serve as an important reference for all clinicians, researchers and patients who are interested in the subject of varicoceles. In time, we hope that some of the readers will actually contribute their own creative ideas about this interesting subject. Please, enjoy the book.

Multiple Choice Questions and Answers

1. Varicoceles have been managed from the first to nineteenth centuries AD for the treatment of pain alone. This was known as the "Pain Era." The following techniques were used for this purpose of pain relief, except one. Please identify this exception:
   (a) Scrotal surgery whereby the enlarged internal veins were ligated and the superficial veins were cauterized.
   (b) Scrotal surgery whereby a section of the scrotum was removed and then the scrotum was re-sutured to elevate the scrotal contents.
   (c) **Inguinal surgery that exposed and removed the veins of the spermatic cord.**
   (d) Use of a scrotal support.
2. The first varicocelectomy for the treatment of male infertility was performed in 1952. This latter era has been known as the "Fertility Era." Since that time, the following techniques were used for the treatment of male infertility, except one. Please identify this exception:
   (a) Microsurgery and selective occlusion of the veins of the spermatic cord.
   (b) **Laser occlusion of internal spermatic veins.**
   (c) Percutaneous venography and occlusion.
   (d) Laparoscopy and occlusion of the internal spermatic veins above the internal inguinal ring.
3. The basis for the "pathophysiology" of varicoceles includes several factors, except one. Please identify the exception:
   (a) **Increased metabolites from the adrenal glands.**
   (b) Increased sperm DNA damage.
   (c) Reactive oxygen species from the walls of the dilated internal spermatic veins.
   (d) Increased testicular heat as a result of internal spermatic vein backflow.
4. Some clinicians recommend diagnosis and treatment of varicoceles among adolescents for the following reasons, except one. Please identify this exception:
   (a) The "fertility" effect may be progressive into adulthood.
   (b) Varicoceles are correctable, even in adolescents.
   (c) **The fertility status of each adolescent must be determined by a semen analysis.**
   (d) Adolescents may present with reduction of testicular growth.
5. Correction of a varicocele may improve the following, except one. Please, identify the exception:
   (a) Correction of a varicocele may improve the results of IVF for couples who require assisted reproduction to achieve a pregnancy.
   (b) Ageing males with low serum testosterone and a varicocele may benefit from a varicocelectomy because the repair may restore a normal serum testosterone.
   (c) Following a varicocelectomy, some men remain infertile, but the sperm density may improve sufficiently for success with IUI.
   (d) **The varicocelectomy may improve sleep patterns.**

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2 Anatomic Theories of Varicocele Origin

Neel Parekh and Edmund Sabanegh Jr.

Key Points

- Since there is substantial collateral arterial blood flow, the internal spermatic artery may be divided during varicocele repair without impairment of testis perfusion because of intact collateral arterial supply.
- The typical anatomic pattern of the left internal spermatic vein is to drain into the left renal vein lateral to the vertebral column. The right internal spermatic vein usually terminates into the inferior vena cava just below the right renal vein.
- Countercurrent heat exchange is thought to be the method by which arterial blood to the testis is cooled 2–4 °C lower than core body temperature, which is necessary for normal spermatogenesis.
- There are multiple proposed theories that contribute to varicocele formation (elevated hydrostatic pressure, valvular mechanisms, and the nutcracker phenomenon), but they are controversial and the etiology may be multifactorial.
- Patients may present with unilateral or bilateral varicoceles, but left-sided varicoceles are the most prevalent.

Introduction: Definition and History of Varicoceles

Varicocele is defined as a vascular abnormality resulting in the palpable enlargement and elongation of the testicular pampiniform venous plexus within the spermatic cord. Varicoceles are cited as the most common cause of male infertility worldwide. Studies denote an overall prevalence of 15–20% in the healthy adult male population [1, 2]. In patients being evaluated at a male infertility clinic, varicocele is identified in 21–41% of men with primary infertility and 75–81% of those with secondary infertility [3]. Furthermore, the World Health Organization reviewed semen analyses from 9043 men, 25.4% of those with abnormal semen analyses and 11.7% with normal semen analyses had varicoceles [4]. Typically, left-sided varicoceles are noted in 90% of cases and 10% occur bilaterally. A solitary right-sided palpable varicocele is seen in less than 1% of patients.

N. Parekh (✉)
Glickman Urologic and Kidney Institute, Cleveland Clinic, Cleveland, OH, USA
e-mail: parekhn3@ccf.org

E. Sabanegh Jr.
Executive Administration, Cleveland Clinic, Cleveland, OH, USA

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In 1843, British surgeon T.B. Curling first utilized the term varicocele to delineate the abnormal dilation of veins in the spermatic cord. He proclaimed "decreasing powers of the gland" to highlight varicoceles association with infertility [5]. A variety of surgical methods were developed to repair varicoceles for pain in the nineteenth century. It was not until W.S. Tulloch published the results of his case series in 1955 that the relationship between varicocele and infertility was recognized. Tulloch published a case series of 30 men who had undergone varicocele repair (unilateral or bilateral); 26 of his patients demonstrated improvement in semen parameters and 10 yielded successful fecundity [6]. Despite the extensive body of literature concerning varicoceles, there remains significant controversy regarding their development and pathophysiology. The purpose of this chapter will be to provide an overview of the basic anatomy of varicoceles and discuss prominent theories which may explain their origin.

Anatomy

Arterial blood flow to the testis emanates from three significant sources: the internal spermatic (testicular or gonadal) artery, the deferential (vasal) artery, and the cremasteric (external spermatic) artery. The internal spermatic artery is derived from the abdominal aorta just below the level of the renal artery and is the main blood supply to the testes. Its diameter is larger than the total diameter of the combined deferential and cremasteric arteries [7]. The deferential artery arises from the superior vesicle artery via the internal iliac (hypogastric) artery [8]. Lastly, the cremasteric artery arises from the inferior epigastric artery at the level of the internal inguinal ring where it joins the spermatic cord. The cremasteric artery primarily supplies the tunica vaginalis and anastomoses with the other arteries at the testicular mediastinum. Angiographic studies demonstrate that in 56% of patients, there is a single internal spermatic artery [9]. However, due to substantial arterial interconnections, the internal spermatic artery may be divided during varicocele repair without impairment of testis perfusion because of intact collateral arterial supply (Fig. 2.1).

Similar to the arterial supply of the testis described above, the venous system forms multiple anastomoses at the mediastinum of the testis. This is called the pampiniform plexus, which is formed by three groups of veins: the anterior, middle, and posterior veins. The anterior spermatic veins travel alongside the internal spermatic artery and at the level of the superficial inguinal ring; coalesce into three or four veins and travel into the pelvis. These branches eventually coalesce to form a single internal spermatic vein at the level of the internal inguinal ring before terminating into the inferior vena cava on the right and the renal vein on the left. The middle deferential veins accompany the vas deferens to drain via the internal iliac vein. The posterior spermatic veins traverse alongside the spermatic cord and empty into the external pudendal and cremasteric veins. Based on the results of an intraoperative surgical venography study performed by Wishahi [10], venous drainage of the testes is mainly via the internal spermatic vein, followed by the external pudendal, deferential, and cremasteric veins in decreasing order of significance. However, there remains preservation of venous return of blood from the testicles with internal spermatic vein ligation during varicocelectomy through the collateral supply between the middle and posterior veins.

The typical anatomic pattern of the left internal spermatic vein is to drain into the left renal vein lateral to the vertebral column [11]. The right internal spermatic vein usually terminates into the inferior vena cava just below the right renal vein. Variations in these findings were found quite frequently in studies examining cadaver specimens [12]. Most commonly, the classic anatomic configuration described above is seen on the right in 78% of patients and on the left in 79% of patients. Anomalous drainage patterns described on the right include termination of the testicular vein in the renal vein in 8% and multiple veins terminating in the inferior vena cava (IVC) and the renal vein in 16%. Anomalous drainage patterns described on the left include multiple veins terminating in the renal vein in 20%. Infrequently, one of multiple branches may terminate in the infrarenal IVC [11, 13].

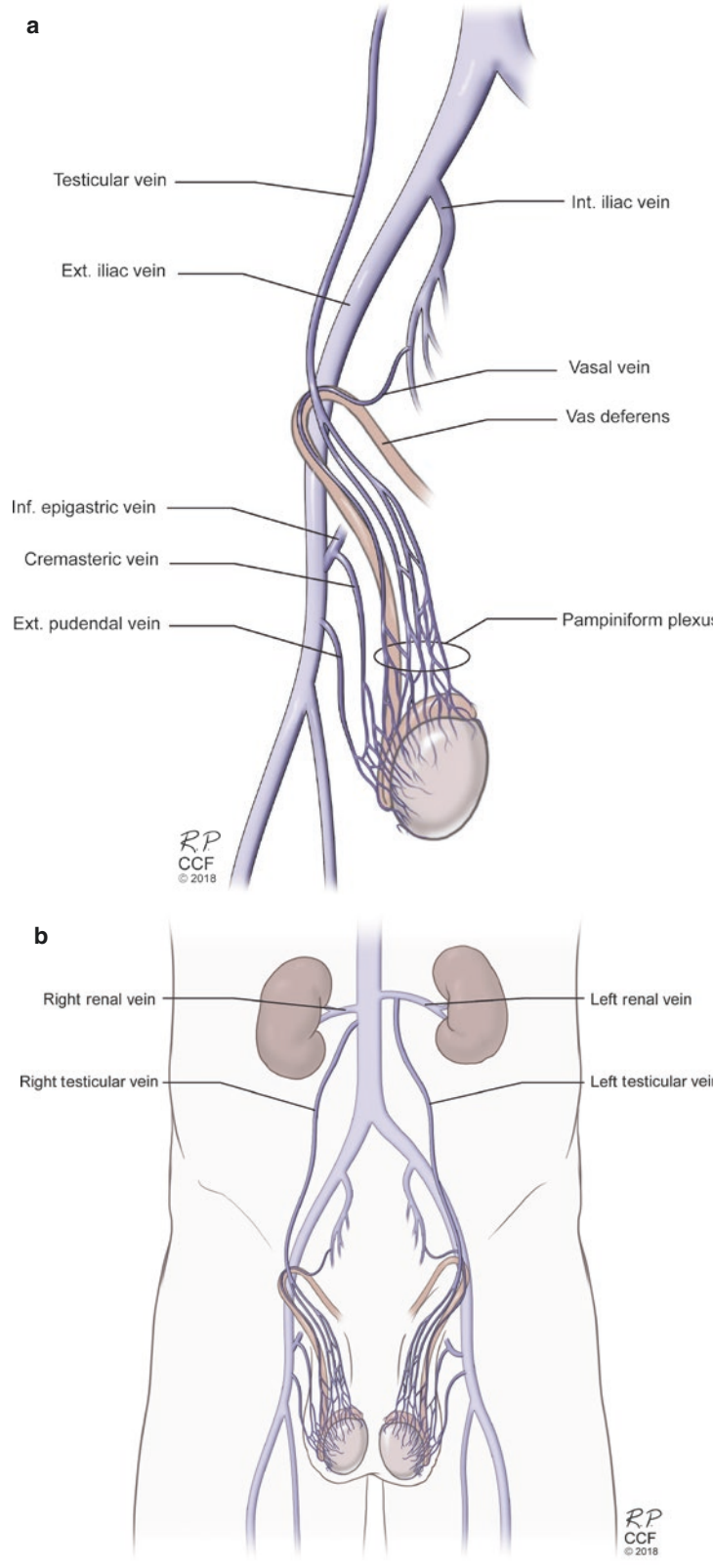

Fig. 2.1 Anatomic illustration of the testicular venous system (**a**), and typical drainage pattern of the right internal spermatic vein into the IVC and the left internal spermatic vein into the left renal vein (**b**). (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2018. All Rights Reserved)

The pampiniform plexus traverses through the inguinal canal surrounding the testicular arteries, lymphatics of the spermatic cord and the vas deferens. This close association between the pampiniform plexus and the arterial supply to the testis allows for countercurrent heat exchange, which is thought to be the method by which the arterial blood in the spermatic cord is cooled [14, 15]. The returning venous blood absorbs heat thereby supplying arterial blood to the testis that is 2–4 °C lower than the rectal temperature in normal men which is necessary for normal spermatogenesis [16]. Similarly, small molecules, such as testosterone, are able to passively diffuse in a concentration-limited manner from the veins to the artery [17]. Stasis of venous blood, like which is seen with varicoceles, may mitigate the transfer of testosterone into testicular and epididymal cells which is required for healthy spermatogenesis. Varicoceles and cryptorchidism play a role in the loss of the temperature gradient created by this system and are associated with testicular dysfunction in men [16].

Theories of Varicocele Origin

Hydrostatic Pressure

Patients may present with unilateral or bilateral varicoceles, but as discussed previously, left-sided varicoceles are the most prevalent. Although controversial, there are a variety of anatomic observations that are believed to play a key role in the theory of varicocele origin (Fig. 2.2). First, the left internal spermatic vein empties into the renal vein while the right internal spermatic vein drains directly into the inferior vena cava. The left internal spermatic vein is approximately 8–10 cm longer than the right internal spermatic vein. This results in greater venous pressure on the left which is subsequently transferred to the pampiniform plexus resulting in dilation [18–21]. In adolescents, Delaney et al. [22] shared their observations in a study of 43 patients diagnosed with varicoceles. These patients were noted to be taller than their age-matched controls. The authors elucidated that taller patients may have a longer spermatic vein which results in increased hydrostatic pressure and subsequent internal spermatic vein dilation [22]. Next, the left spermatic vein drains into the left renal vein at an approximately 90 degree angle, while the right spermatic vein inserts more obliquely. This causes the left internal spermatic vein to be subject to elevated hydrostatic pressures within the left renal vein. The opposite effect is noted with the right internal spermatic vein and its oblique insertion, which protects it from pressure variations from the inferior vena cava [23]. Finally, the inferior vena cava is subject to considerably greater blood flow compared to the left renal vein, which is believed to improve the venous flow of the right internal spermatic vein compared to that of the left by the Venturi principle [24]. The overall effect of these anatomic elements is increased hydrostatic pressure in the left internal spermatic vein in relation to the right internal spermatic vein, which clearly predisposes the left side to varicocele formation [25]. A 1980 study by Shafik and Bedeir [19] measured pressure elevations and the relationship to varicocele formation. They analyzed venous tension patterns in spermatic cord veins in 32 patients with a left-sided varicocele and 30 normal individuals using a saline manometer. The authors determined that in the varicocele group there were higher venous pressure levels within the left internal spermatic vein which is consistent with the theory of increased hydrostatic pressure predisposing to varicocele formation [19].

Valvular Mechanisms

Another theory of varicocele origin is the belief that the spermatic veins have incompetent or absent valves that predispose to varicocele formation. Doppler ultrasound and venography studies have demonstrated two different pathophysiologic patterns: stop-type and shunt-type varicoceles [26]. These two subtypes of varicoceles are classified based on the level of the valvular abnormality in relation to the communicating veins (internal spermatic, external spermatic (cremasteric), vasal and external pudendal

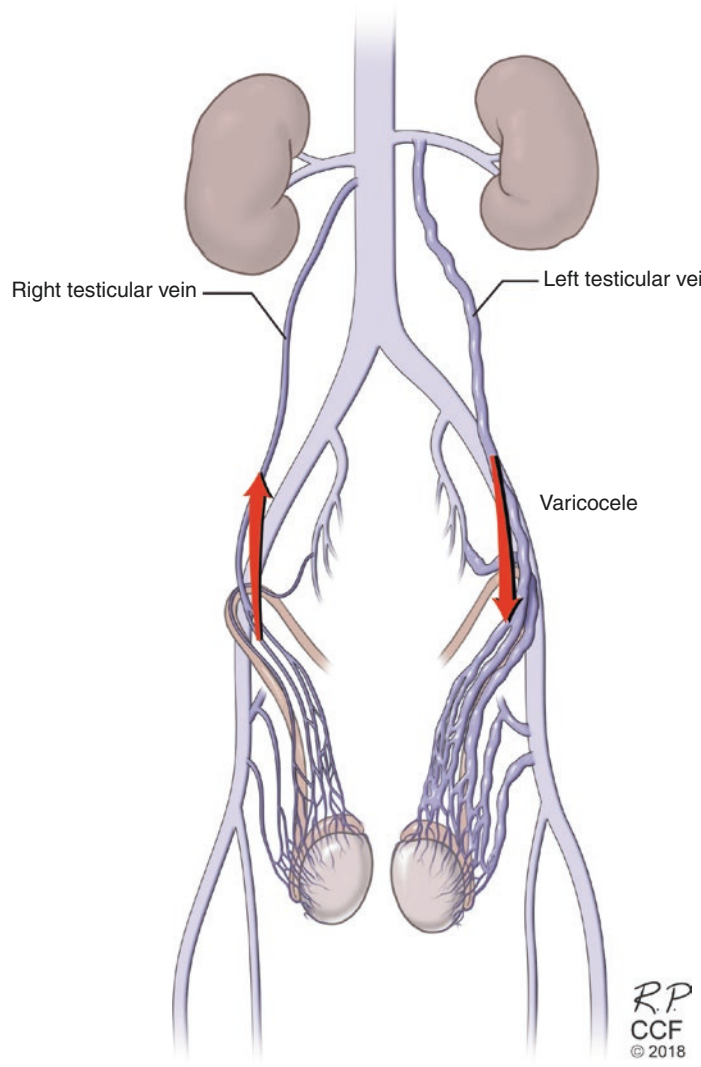

Fig. 2.2 Illustration depicting effect of increased hydrostatic pressure and resultant distension of the pampiniform plexus. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2018. All Rights Reserved)

veins). However, this concept is controversial as absence of valves has been noted in men without varicoceles on autopsy. Ahlberg et al. [27] identified absent valves in approximately 40% of left spermatic veins and 23% of right spermatic veins (Fig. 2.3).

The shunt-type varicocele has been identified in approximately 85% of patients. It is defined by incompetent valves below the level of communicating vessels. Consequently, there is uninterrupted retrograde flow from the internal spermatic vein into the pampiniform plexus and orthograde drainage into the external spermatic and vasal veins. The flow of venous blood from the internal spermatic vein to the external spermatic veins causes dilation of both venous systems which can predispose patients to larger varicoceles. Shunt-type valves are widely distributed and abundant throughout the venous system, therefore surgical management is considered to be less effective. Mohseni et al. [28] echoed these findings in a study of 74 children and adolescents with either shunt-type or stop-type varicoceles. They determined that there was a higher incidence of testicular hypotrophy with the shunt-type compared to the stop-type varicoceles. Furthermore, they reported a higher recurrence rate for shunt-type varicoceles that were surgically managed

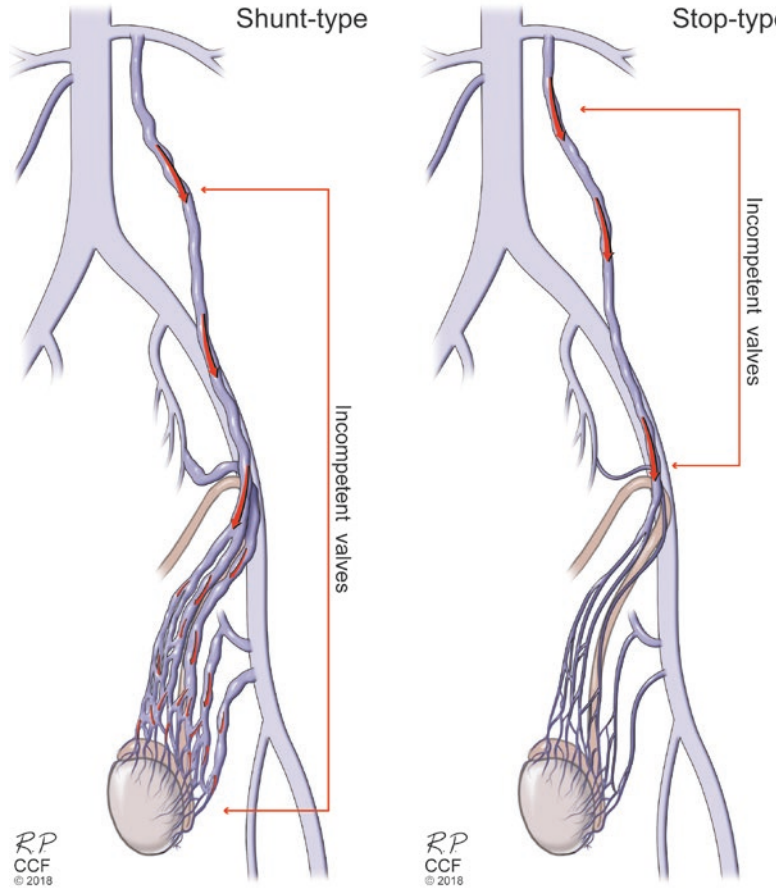

Fig. 2.3 Shunting through the communicating veins resulting in a shunt-type varicocele (**a**), and a stop-type varicocele where there are competent venous valves above the level of communicating veins (**b**). (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2018. All Rights Reserved)

through a retroperitoneal approach as opposed to an inguinal approach.

The stop-type varicocele is a result of competent venous valves above the level of communicating veins. This subtype has been identified in approximately 15% of varicoceles [26]. It allows for transient reflux to occur from the internal spermatic vein into the pampiniform plexus, but the reflux towards the communicating veins is hindered by a competent valve above the level of communicating veins. Typically, only the internal spermatic vein is found to be dilated. However, it is unclear if it is in fact the venous dilation that results in the incompetent valves or vice versa. Regardless, ligation of the stop-type varicocele should successfully resolve the varicocele by mitigating the refluxing components of the venous drainage system [26].

More recently, Yasim et al. [29] described an association between varicose veins of the lower extremities and varicoceles. One-hundred patients undergoing surgical repair of varicose veins were included in the study of which 72 of the patients were found to have varicoceles. Doppler ultrasound identified varying grades of reflux flow in the majority of these patients. The authors proposed that incompetent venous valves play a significant role in both disease processes. Similarly, Levinger et al. [30] reported an increasing prevalence of varicocele with age, with an incidence of 75% in the eighth decade of life. Through the use of fluid-mechanics analysis they cite that systemic venous insufficiency may be a mediator for both varicocele and lower extremity venous incompetence.

Nutcracker Phenomenon

Another mechanism that may result in varicocele formation is the nutcracker effect, also known as left renal vein entrapment syndrome. This phenomenon refers to the anatomic compression of the left renal vein by the abdominal aorta and superior mesenteric artery or common iliac artery [9, 14, 20, 31]. Subsequent venous stasis in the left renal vein produces pressure elevations that are transferred to the left internal spermatic vein and pampiniform plexus [32]. Varicoceles almost always occur on the left side, but the exact prevalence of the nutcracker effect is not known [33–35] (Fig. 2.4).

The gold standard for diagnosis is through the measurement of the pressure gradient between the left renal vein and inferior vena cava via selective left renal venography. The left renal vein typically measures 6–10 cm in length and the mean normal left renal vein diameter is 4–5 mm [36]. Normally, the pressure gradient between the left renal vein and inferior vena cava is less than or equal to 1 mmHg. In order to diagnose the nutcracker effect, the pressure gradient between the left renal vein and inferior vena cava should be greater than 3 mmHg. Given the invasiveness of venography, several studies have demonstrated the combination of B-mode ultrasound measurement of the diameter of the left renal vein and Doppler ultrasound measurement of the left renal vein peak velocity as useful in establishing the diagnosis of the Nutcracker effect [37, 38]. Imaging may demonstrate dilatation of the left renal vein even in asymptomatic cases. Therefore, in order to successfully diagnose this phenomenon, ratios for means peak velocities and diameters of the lateral: medial left renal veins are 4–5 and 1.5–5 mm, respectively [37–40].

Unlu et al. [41] prospectively utilized abdominal and scrotal ultrasound in 35 patients with and without varicoceles. Doppler ultrasound was performed with patients' supine, during Valsalva and in the erect position. In the varicocele group, changes in the left renal vein peak velocity and

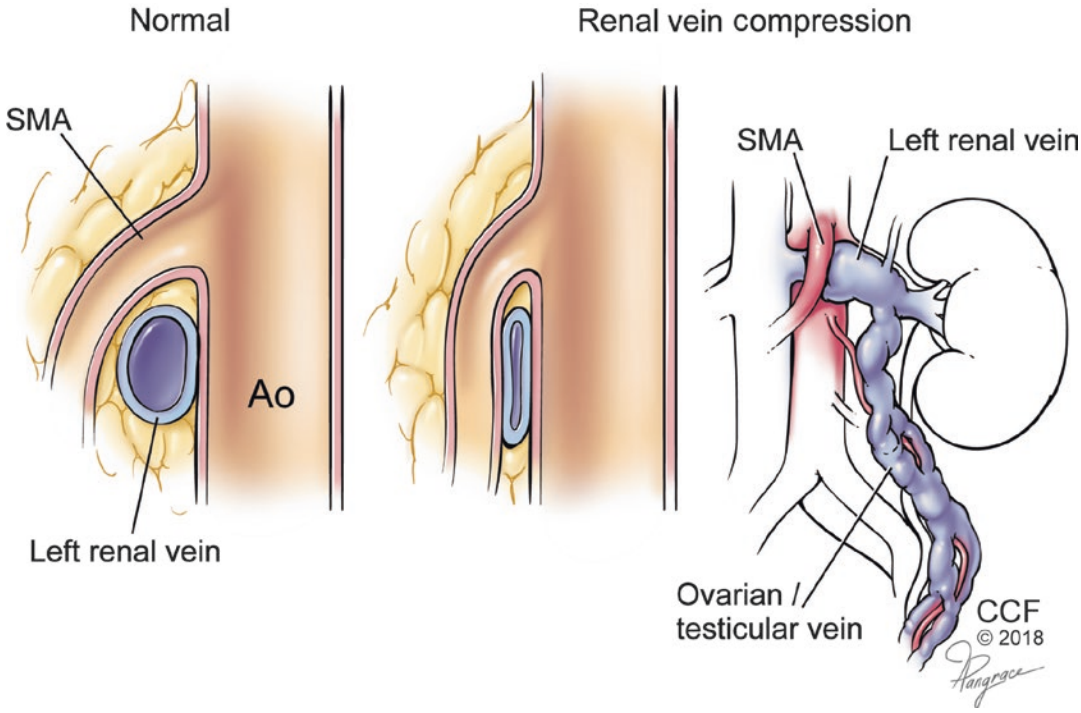

Fig. 2.4 Nutcracker phenomenon (left renal vein entrapment syndrome). (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2018. All Rights Reserved)

diameters were consistent with compression in virtually all patients, particularly in the erect position and with Valsalva. This may be related to visceral ptosis and a pressure gradient while in the erect position [42]. In the pediatric population, the nutcracker effect may be more prevalent due to diminished retroperitoneal fat and narrowing of the aortomesenteric angle. However, in children, the use of Doppler ultrasonography may be inadequate when compared to adults [35, 40]. In the rare instance of an isolated right-sided varicocele in an older patient, classic teaching suggests prompt work up with the use of abdominal imaging to rule out external compression from a retroperitoneal mass; however, this has been challenged by a recent retrospective review of 337 men with right-sided varicocele [43]. DeWitt et al. determined that an association with malignancy was not significantly different in right-sided varicoceles, with rates similar to left-sided and bilateral varicoceles [43].

Conclusion

The anatomic cause of varicocele formation is controversial and likely multifactorial. Understanding the basic anatomic principles of varicoceles is of utmost importance, particularly when attempting to determine the pathophysiologic etiology (e.g. hyperthermia, oxidative stress, reflux of toxic metabolites, etc.) of testicular dysfunction and infertility. These pathophysiologic theories will be covered extensively throughout this book. Furthermore, the clinician must be familiar with the variability in varicocele anatomy as it may aid in guiding patient management and potential surgical approaches.

Review Criteria

We extensively searched Google Scholar, PubMed, Medline, Clinical Key and ScienceDirect for articles focusing on the anatomy, theories of origin, and pathophysiology of varicoceles.

We began our literature search during February 2018 and completed it by May 2018. The following key words were utilized in our search: "varicocele," "varicocele anatomy," "varicocele incidence," "etiology of varicocele formation," "varicocele pathophysiology," "infertility," and "varicocele repair." We reviewed only English language articles. Illustrations were created with assistance from an institution-based artist.

Multiple Choice Questions and Answers

1. The most commonly seen drainage pattern of the internal spermatic vein is:
   (a) **Right-IVC, Left-Left Renal Vein**
   (b) Right-IVC, Left-IVC
   (c) Right-Right Renal Vein, Left-Left Renal Vein
   (d) Right-Right Renal Vein, Left-IVC
2. The ideal temperature for normal spermatogenesis is:
   (a) About 0–1 degrees below body temperature
   (b) **About 2–4 degrees below body temperature**
   (c) About 5–6 degrees above body temperature
   (d) About 2–3 degrees above body temperature
3. Which of the following is not a proposed theory of testicular dysfunction in men with varicocele?
   (a) Heat Stress
   (b) Reactive Oxygen Species
   (c) Retrograde flow of Toxic Metabolites
   (d) **Testicular Hypothermia**
4. Which of the following arteries does not provide blood supply to the testicle?
   (a) Deferential
   (b) Internal Spermatic
   (c) **External Pudendal**
   (d) Cremasteric

5. Which of the following theories is not considered a potential cause of varicocele formation?
   (a) Left Renal Vein Entrapment Syndrome
   (b) Incompetent Venous Valves
   (c) **Enlarging Hydrocele**
   (d) Hereditary Factors

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3 Scrotal Hyperthermia, Hormonal Disturbances, Testicular Hypoperfusion, and Backflow of Toxic Metabolites in Varicocele

Ahmad Majzoub, Chak-Lam Cho, Ashok Agarwal, and Sandro C. Esteves

Key Points

- In varicocele patients, dilatation of the pampiniform plexus leads to venous stasis and retrograde blood flow which alters the heat exchange mechanism leading to testicular hyperthermia.
- Testicular hyperthermia can cause spermatogenic dysfunction by altering the DNA synthesis enzymes function, gene expression, and protein synthesis.
- Animal and human studies have illustrated an elevation of intra-testicular temperatures in relation to varicocele.
- Decreased testosterone concentrations seen in infertile men with varicocele may be caused by some degree of Leydig cell dysfunction occurring secondary to varicocele.
- Elevated venous pressure may alter the intratesticular oncotic and hydrostatic pressures changing the paracrine environment of key hormones and influencing fluid exchange.
- Controversy still surrounds the pathophysiologic effect of retrograde flow of renal/adrenal metabolites on testicular function.

Introduction

Varicocele is the abnormal dilatation and reflux of blood in the pampiniform plexus of veins draining the testicles. Varicocele has long been considered a controversial subject in the field of andrology specifically regarding why, when, and to whom varicocelectomy can be applied. While it is a common cause of male infertility found in about 40% and 80% of men with primary and secondary infertility respectively, it is also present in up to 20% of the general male population, many of whom are able to reproduce naturally [1, 2]. Many experts believe that the surgical repair of varicocele should be applied only in a meticulously selected group of infertile men, although there are no generally accepted criteria. So far, the only confirmed prognostic factor for

A. Majzoub (✉)
Department of Urology, Hamad Medical Corporation, Weill Cornell Medicine Qatar, Doha, Qatar

C.-L. Cho
S.H. Ho Urology Centre, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

A. Agarwal
American Center for Reproductive Medicine, Department of Urology, Cleveland Clinic, Cleveland, OH, USA

S. C. Esteves
ANDROFERT, Andrology & Human Reproduction Clinic, Campinas, SP, Brazil

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achievement of pregnancy after varicocelectomy is the age of the female [3]. It is therefore imperative to understand the pathophysiology of infertility in men with varicocele to better explain this association and aid in selecting patients who would benefit from treatment.

The etiology of varicocele is not fully explained. According to one theory, varicocele is the result of anatomical differences between right and left spermatic veins [4]. In fact, the right internal spermatic vein is embedded directly into the inferior vena cava at an acute angle, while the left internal spermatic vein is embedded into the left renal vein at a right angle. It is believed that this discrepancy leads to an increase in the hydrostatic pressure of the left spermatic vein, which is consequently transferred to the spermatic venous plexus leading to its dilation [1]. A second theory is based on the fact that internal spermatic veins lack functional valves, which can result in regression of blood. Finally, a third theory suggests that there is a partial impediment of the left spermatic vein due to the compression of the left renal vein between the aorta and the upper mesenteric artery ("the nutcracker phenomenon") [1]. Nevertheless, the adverse effect of varicocele on spermatogenesis can be assigned to many factors such as an elevation of testicular temperature, increased intra-testicular pressure, hypoxia associated with altered blood flow, reflux of toxic metabolites from the adrenal glands, and imbalanced hormonal profile (Fig. 3.1). This chapter serves to highlight the various pathophysiologic mechanisms interrelating varicocele to male infertility soliciting the available evidence supporting each association.

Scrotal Hyperthermia

The anatomic position of the testes within the scrotal sac along with the countercurrent heat exchange mechanism accommodated by the pampiniform plexus of veins is pledged for testicular temperature regulation [5]. With this characteristic phenomenon, the inflowing arterial blood flow is cooled down by the outflowing venous blood of the pampiniform plexus thereby maintaining a scrotal temperature few degrees below the core body temperature that is favorable for optimal testicular function [6]. However, in varicocele patients, dilatation of the pampiniform plexus leads to venous stasis and retrograde blood flow which alters the heat exchange mechanism leading to testicular hyperthermia. Such elevation in scrotal temperature in varicocele patients is believed to be the principal factor contributing to abnormal sperm physiology [1, 5, 7].

Testicular hyperthermia can alter the function of a number of enzymes responsible for DNA synthesis, as those enzymes often exhibit optimal activity at temperatures lower than the body's core temperature [8–10]. Enzymes such as Topoisomerase 1 and DNA polymerase that are

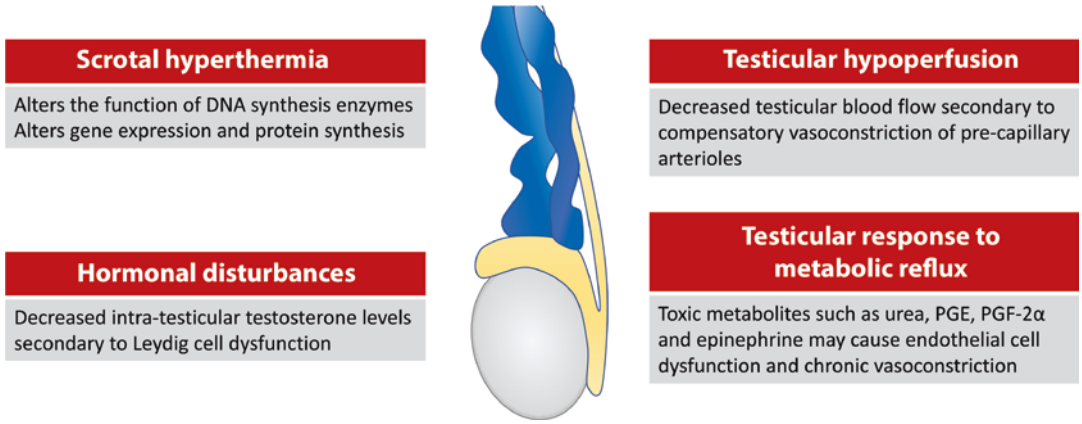

Fig. 3.1 The pathophysiologic mechanisms linking varicocele with male infertility

principally involved in DNA synthesis were downregulated in patients with varicocele [9, 11]. Gene expression and protein synthesis have also been found to be influenced by testicular temperature. De Amicis et al. determined that the varicocele sperm exhibited reduced expression of phosphatidylinositol 3-kinase (PI3K), a protein responsible for sperm capacitation, acrosome reaction, and fertilization, compared with normal sperm [10]. The authors also depicted changes in the distribution of PI3K enzyme along the sperm cell which was only detected in the head of varicocele sperm in comparison to the head, nucleus, and entire tail of normal sperm. Hosseinifar et al. compared the profile of sperm protein expression between men with and without varicocele revealing decreased expression of heat shock proteins, mitochondrial proteins, and cytoskeleton proteins in patients with varicocele [12]. Another study utilizing semi-quantitative real-time polymerase chain reaction analysis of ejaculated sperm detected dramatic reduction in the expression of HSPA2 gene, a gene that encodes for heat shock protein 2, in adolescents with varicocele and oligozoospermia compared to adolescents without varicocele and normal sperm concentration [13]. ATP5D, another gene that encodes for a segment of the mitochondrial adenosine triphosphate synthase (ATPase) which provides energy necessary for sperm flagellar motion was found to be downregulated in men with varicocele [12]. These findings highlight the vulnerability of the testes to elevated temperature altering the expression of genes responsible for normal sperm physiologic function. However, it appears sensible to demonstrate testicular temperature elevation that occurs in the context of varicocele. Several studies have investigated this particular conjecture with controversial results that were mainly caused by varying temperature measurement techniques (scrotal or intratesticular).

Using a surface probe, Goldstein and Eid demonstrated increased scrotal skin temperatures of infertile men with varicocele in comparison to normal controls [14]. This result was similar to an earlier study utilizing a water-bath thermometer to measure the scrotal surface temperature [5]. Physical activity appears to inflict wide variation in scrotal temperature as noted by studies utilizing continuous portable digital temperature recorders in normal men over a 24 h period. Despite recording meniscal elevations in scrotal temperature in varicocele patients compared with normal controls, no changes in temperature were detected after performing varicocelectomy for the patient group [15]. Salisz et al. measured the surface scrotal temperature in adolescents with grade 2–3 varicocele in both the supine and standing positions comparing them to controls. The authors noted an overall bilateral increase in scrotal temperature with further increase ipsilaterally, after standing, in varicocele patients compared to controls. Adolescents who were unable to maintain a left scrotal temperature at least 1.4 °C cooler than axillary temperature in the standing position were noted to have the most significant reduction in testicular volume [16].

On the other hand, other studies failed to find a direct relationship between varicocele and elevated scrotal skin temperature. For instance, a study done by Mieusset et al. showed no difference in scrotal temperature when comparing men with and without varicocele [17]. Similarly, Lund and Nielsen showed that although varicocele was associated with impaired sperm quality compared with a control group, no differences in scrotal skin temperature were noted between men with and without varicocele [18].

Intratesticular temperature measurements were most commonly conducted in animal studies. Results have revealed an increase in bilateral intratesticular temperature following ipsilateral iatrogenic varicocele formation [19, 20] and normalization of temperature following varicocele repair [21, 22].

Goldstein and Eid measured intratesticular temperature in humans through inserting a needle thermistor 1 cm into the testicular substance and found bilateral elevation of intratesticular temperature in patients with unilateral varicocele [14]. Others demonstrated the return of intratesticular temperature elevation to control levels in varicocele patients following surgical repair [23].

In an undergoing study, we utilized an infrared digital thermography camera (FLIR E5, FLIR

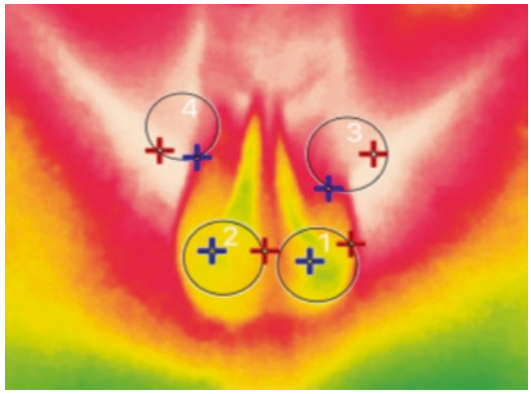

Fig. 3.2 Infrared image of scrotal region using FLIR E5 camera. Surface temperatures are captured as seen in the circles and are reported as min, max, and average

Systems Inc., Watsonville, USA) to measure scrotal surface temperatures in infertile patients with varicocele, fertile men with varicocele and a normal control group [not published] (Fig. 3.2). Data of 73 infertile varicocele patients and 32 fertile varicocele men is available revealing higher, though insignificant, left testicular temperature readings in infertile varicocele patients compared with fertile varicocele men (33.4 ± 0.8 °C vs. 32.9 ± 0.8, $p = 0.23$). Further insights still await the inclusion of a normal control group.

Although the data showing a clear association between scrotal hyperthermia and varicocele is still conflicting, animal and human studies have illustrated the elevation of intra-testicular temperatures in relation to varicocele.

Hormonal Disturbances

Intratesticular testosterone levels are crucial for normal spermatogenesis. It has been postulated that the decreased testosterone concentrations seen in infertile men with varicocele may be caused by some degree of Leydig cell dysfunction occurring secondary to varicocele and attributing to the development of infertility.

This theory has been concurred by a number of animal studies which identified a decline in serum testosterone levels following the development of experimental varicocele [20]. While some studies reported ipsilateral reduction of intratesticular testosterone levels following varicocele induction, others observed a bilateral effect with reduction in enzymes responsible for testosterone biosynthesis (17,20-desmolase and 17a-hydroxylase) [24]. The reduction of intratesticular testosterone in varicocele has been also explained by an attenuated response to human chorionic gonadotrophin (HCG) stimulation or by decreased binding of HCG to Leydig cell receptors in varicocelized animals [22, 25]. These findings suggest that varicocele could impair testosterone biosynthesis resulting in decreased serum and intratesticular concentrations.

A multicenter study conducted by the World Health Organization in 1992 examining the influence of varicocele on fertility parameters reported lower mean testosterone concentrations in varicocele patients who were older than 30 years compared with younger patients suggesting a negative, time-dependent effect of varicocele on the function of Leydig cells [26]. Zalata et al. demonstrated a direct correlation between androgen receptors and sperm count, motility and morphology and reported a sharp decline in androgen receptor expression in infertile patients with varicocele compared to infertile men without varicocele [27]. On the other hand, several studies indicated no difference in FSH, LH, testosterone, and estradiol concentrations in both peripheral and testicular venous blood in men with and without varicocele [28–31], while others showed no difference in testosterone concentration before and after varicocele repair [32, 33]. Despite the decrease in testosterone production that may occur with varicocele patients, many have values that lie within the normal reference range secondary to some degree of Leydig cell hyperplasia which compensates for the decreased testosterone production [34].

Hudson and Mckay suggested that Leydig cell function may be more accurately measured with gonadotrophin response to gonatotrophin-releasing hormones (GnRH) rather than the human chorionic gonadoropin (HCG) stimulation test [31]. Men with varicocele demonstrated exaggerated response in terms of FSH and LH release, to GnRH after a 4-h infusion. This response was even larger with severely oligozoospermic men

than those with sperm count ranging between 11 and 30 x 10^6/ml. Predominantly men with exaggerated gonadotrophin response to GnRH were likely to have more improvement in their semen parameters following varicocelectomy regardless of the degree of oligozoospermia [31]. Others illustrated that a normal LH response to GnRH stimulation post varicocelectomy was associated with improved fertility postoperatively [35].

These findings advocate that varicocele affects the hypothalamic pituitary gonadal axis, and establish the fact that men with varicocele and abnormal Leydig cell function may benefit the most from varicocelectomy.

Testicular Hypoperfusion

Blood pressure in pre-capillary arterioles and post-capillary venules in the testes is low compared to other body tissues. Hence, even minimal changes in blood pressure can affect the testicular environment. The increased venous tension that occurs with varicocele may result in compensatory vasoconstriction of the pre-capillary arterioles to maintain normal intratesticular pressure homeostasis [36]. Direct measurements of the intravascular pressures in micro-vessels located on the subcapsular surface of hamster testis demonstrated significantly lower testicular capillary pressure. Furthermore, the vascular resistance distribution shows that capillary pressure may be dramatically sensitive to increases in venous pressure in the hamster model [37].

In humans, the normal resting venous pressure of the spermatic veins was measured through the introduction of a needle into a pampiniform vein and attaching it to a saline manometer [38]. The recorded ipsilateral venous pressure of varicocele patients was found to be higher than control subjects. Despite this, more than half of the varicocele patients had normal semen parameters, questioning whether there is a relationship between increased venous pressure and impaired spermatogenesis [3].

Nevertheless, the same authors assessed venous pressure changes following varicocelectomy and reported a reduction in pressure readings in 88% of 60 patients after surgery. This reduction in venous pressure was associated with improvement in semen parameters in 70% of patients and with the production of natural pregnancy in 32% of patients. A greater improvement in sperm motility was observed in patients who were vs. those who were not able to conceive naturally yet demonstrating a decrease in venous pressure postoperatively [39].

The increase in venous pressure is believed to result in downregulation of arteriolar blood flow which may have pernicious effects on the nutrient supply of the testes, and as a result may affect spermatogenesis. Furthermore, the elevated venous pressure may alter the intratesticular oncotic and hydrostatic pressures changing the paracrine environment of key hormones and influencing fluid exchange.

Proof that this sentiment may be true is the reduced adenine nucleotide concentrations and nicotinamide adenine dinucleotide-cytochrome C-reductase activity in varicocele-bearing rats' testes as opposed to those in sham-operated rats as shown in few studies [40, 41] suggesting defective energy production in varicocele-induced testes.

Lee et al. observed increased levels of hypoxia-inducible factor 1-alpha (HIF 1-alpha) in testicular veins of men diagnosed with varicocele, confirming that cells of the testicular microenvironment are exposed to less oxygen delivery [42]. HIF plays a critical role in the promotion of cell survival in hypoxic conditions. It promotes the biosynthesis of new vessels when low oxygen levels are present enabling larger quantities of oxygen to reach hypoxic tissues to enhance energy production. Furthermore, HIF also plays a role in programmed cell death also known as cell apoptosis in the presence of a low oxygen environment. Both roles played by HIF whether cell death or cell survival depends on the tissue type and the severity of hypoxia. To study the degree of cell apoptosis, Wang et al. used terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) on experimentally induced varicocele and a control group of rats. They concluded that testicular HIF 1-alpha levels in response to varicocele-induced hypoxia were

associated with increased levels of apoptosis of germ cells thereby suggesting their contribution to male infertility [43].

Testicular Response to Metabolic Reflux

Venous reflux is a common finding observed with venography studies performed on men with varicocele [44, 45]. This finding supports the hypothesis stating that reflux of toxic metabolites from the kidney and suprarenal gland such as urea, prostaglandins E, prostaglandin F 2 alpha and epinephrine may cause endothelial cell dysfunction and chronic vasoconstriction ultimately leading to hypoperfusion and testicular tissue hypoxia [46, 47]. Furthermore, these substances are known to facilitate cellular oxidative stress in multiple human cell cultures in vitro. Supraphysiologic levels of urea, for instance, can induce a state of oxidative stress by reducing the levels of glutathione [48].

Studies exploring the theory at hand have had contradictory results. Retrograde flow of renal/adrenal metabolites was demonstrated by a number of studies [49–51]. MacLeod et al. reported a three-fold increase in the mean catecholamines concentration from testicular venous blood obtained during varicocelectomy in comparison to levels obtained from the peripheral circulation [52]. The authors suggested that the increased catecholamine concentrations may reach the testicular artery through the countercurrent exchange mechanism to impose arteriolar vasoconstriction and tissue hypoxia. On the other hand, studies comparing the levels of other adrenal metabolites such as dehydroepiandrosterone and cortisol between testicular venous blood and the peripheral circulation of infertile men with varicocele failed to find any statistically significant differences [53, 54]. Ito et al. detected an elevation of PGE and PGF levels in spermatic venous blood of varicocele patients. However, they failed to find an elevation in cortisol levels assuming that adrenal metabolites do not reflux contrary to renal metabolites [47]. This, however, was opposed by others who failed to find an increase in renin concentration in spermatic venous blood of varicocele men which should have been raised if renal blood flow was refluxing down the spermatic veins [55].

Retrograde flow of renal/adrenal metabolites has also been dismissed by Sofikitis and Miyagawa who after performing adrenalectomy on varicocelized Wistar rats did not observe any reversal of the varicocele-related pathologic changes including increased testicular temperature, decreased sperm count and motility and decreased testicular weight [22]. Similarly, another animal study in which labeled microspheres infused into the left renal vein did not appear in either testes of animals with induced left varicocele adds to the evidence refuting the metabolic reflux theory [56].

Conclusion

It is not possible to state a single mechanism responsible for varicocele pathophysiology. Hence, the etiology is considered to be multifactorial. It is worth noting that while studying the pathophysiology of varicocele in experimental animal models is indeed useful, the sudden iatrogenic creation of varicocele does not mimic the natural course of this disease in humans nor its detrimental impact on spermatogenesis. On the other hand, human studies are mostly observational, comparing men with and without varicocele, or before and after surgical ligation. While such observations may delineate the effect of varicocele on testicular function, they fail to directly explain the mechanisms involved with such an effect. The available literature highlights the plausible association between varicocele and scrotal hyperthermia, testicular hypoperfusion, hormone dysregulation and reflux of metabolites providing a platform for future investigation to better explain the pathophysiology of varicoceles.

Review Criteria

An extensive search of the literature was done using scientific search engines including Pubmed, Medline, ScienceDirect, and Google Scholar. Search criteria included the following key words: "varicocele," "pathophysiology," "testicular temperature," "oxidative stress," "testicular hypoxia," and "hypothalamic pituitary gonadal axis." Data from published papers or book chapters were included.

Multiple Choice Questions and Answers

1. Testicular temperature is:
   (a) Higher than the core body temperature
   (b) **Lower than the core body temperature**
   (c) The same as the core body temperature
   (d) None of the above
   (e) All of the above
2. Which of the following is TRUE?
   (a) Topoisomerase 1 and DNA polymerase enzymes which are principally involved in DNA synthesis were upregulated in patients with varicocele
   (b) In response to testicular hyperthermia, increased expression of heat shock proteins has been detected in patients with varicocele.
   (c) Physical activity and the individuals' posture has little effect on testicular temperature
   (d) **Although the data showing a clear association between scrotal hyperthermia and varicocele is still conflicting, animal and human studies have illustrated the elevation of intra-testicular temperatures in relation to varicocele.**
3. Intratesticular testosterone:
   (a) Principally originates from the adrenal glands
   (b) Is produced by Sertoli cells under the influence of FSH
   (c) Has little effect on spermatogenesis
   (d) **Maybe normal in patients with varicocele due to Leydig cell hyperplasia**
   (e) Is not influenced by gonadotropin release
4. Which statement is FALSE?
   (a) **The increased venous tension that occurs with varicocele may result in compensatory vasodilatation of the pre-capillary arterioles to maintain normal intratesticular pressure homeostasis**
   (b) Direct measurements of the intravascular pressures in micro-vessels located on the sub-capsular surface of hamster testis demonstrated significantly lower testicular capillary pressure.
   (c) Ipsilateral venous pressure of varicocele patients was found to be higher than control subjects
   (d) Reductions in venous pressure following varicocelectomy have been associated with improvements in semen parameters
   (e) Cells of the testicular microenvironment in varicocele patients are exposed to less oxygen delivery
5. Metabolic reflux in the context of varicocele:
   (a) Is believed to be the principal pathophysiologic mechanism leading to spermatogenic dysfunction
   (b) Is confirmed by the appearance of microspheres in the testes of animals with induced varicocele followed their infusion into the left renal vein
   (c) **Is suggested based on the assumption that reflux of toxic metabolites from the kidney and suprarenal gland such as urea, prostaglandins E, prostaglandin F 2 alpha and epinephrine may cause endothelial cell dysfunction and chronic vasoconstriction ultimately leading to hypoperfusion and testicular tissue hypoxia**
   (d) Is unanimously confirmed by experimental studies
   (e) None of the above

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4 Genetics and Epigenetics of Varicocele Pathophysiology

Viviane Paiva Santana, Cristiana Libardi Miranda-Furtado, and Rosana Maria dos Reis

Key Points

- The variability of clinical phenotypes related to varicocele suggests the presence of multiple genetic, epigenetic, and environmental factors that contribute to the severity of the disease, and may compromise the fertility.
- Chromosomal disorders, mutations, polymorphisms, and epigenetic changes in gene expression have been reported to be associated with varicocele.
- The improvement in sperm count and quality after varicocelectomy is mainly associated with the impact of testicular environment on genetic/epigenetic changes and is not related to somatic chromosomal abnormalities.
- A better understanding of the etiologic origin and aggravating factors of varicocele will facilitate appropriate treatment of varicocele, and several studies are underway to unravel the genetic basis of varicocele.

Introduction

Despite the extensive literature, the relationship between varicocele and male infertility remains unclear. Fertility loss is a striking feature among patients with varicocele, and this is mainly related to reductions in sperm count and quality. Although varicocele is evident in 19–41% of infertile men [1], not all patients with varicocele are infertile or have altered seminal parameters. Understanding the mechanisms that underlie varicocele development is a challenge due to the variety of phenotypes observed, including the degree of venous dilation, differences in seminal parameters, and the possibility of an improvement or a recurrence after varicocelectomy. Thus an array of varicocele phenotypes suggests that a multifactorial and complex mechanism is involved in their development. Genetic alterations, including chromosomal aberrations, genomic instability, gene mutations, and polymorphisms, indicate a predisposition to the development or aggravation of the condition. Moreover, men with family histories of varicocele are at greater risks of developing these anomalies, especially when varicocele is present in first-degree relatives; therefore, genetics may play an important role in its development [2, 3].

Disruption in the testicular environment, such as increased temperature, hypoxia, oxidative stress, and accumulation of toxic

V. P. Santana · C. L. Miranda-Furtado
R. M. dos Reis (✉)
Department of Gynecology and Obstetrics, Ribeirao Preto Medical School of University of Sao Paulo (USP), Ribeirao Preto, SP, Brazil
e-mail: romareis@fmrp.usp.br

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metabolites, often occurs in men with varicocele [4–6]. These alterations may affect the spermatogenesis, leading to poor semen quality due to genetic and epigenetic alterations, since the process of germ cell development is regulated by booth mechanisms (Fig. 4.1). In particular, the epigenetic reprogramming during spermatogenesis, in which the sex-specific pattern of gene expression is established and/or maintained, is strongly affected by environmental factors within the testicle that may contribute to fertility loss as well as the recurrence after treatment of varicocele.

Understanding the mechanisms involved in varicocele pathophysiology and its potential to cause male infertility and other related comorbidities are important to guide the diagnosis and treatment of patients, which will improve the treatment outcomes. Several studies have demonstrated the relationship between varicocele with different genetic and epigenetic abnormalities [7]. In this chapter, we review the main genetic and epigenetic changes related to varicoceles, highlighting the main findings from recent studies that have investigated hereditary or sporadic genetic factors, sperm alterations, environmental factors, and epigenetic modifications and their relationships with the development of the disorder.

Genetic Factors

Genetic abnormalities contribute significantly to failures during spermatogenesis, and they are present in over 15% of infertile men and in 2–8% of men with unexplained infertility and azoospermia or oligozoospermia [8]. Autosomal and Y chromosome genes regulate the development of the male gonads, the urogenital tract, the hypothalamic-pituitary-gonadal axis, and spermatogenesis [9]. Genetic alterations may interfere with the production and maturation of the spermatozoa or they may lead to the production of non-functional gametes. Among the genetic abnormalities associated with varicocele development, the structural and numerical chromosomal abnormalities, gene mutations, polymorphisms, and copy number variations were highlighted.

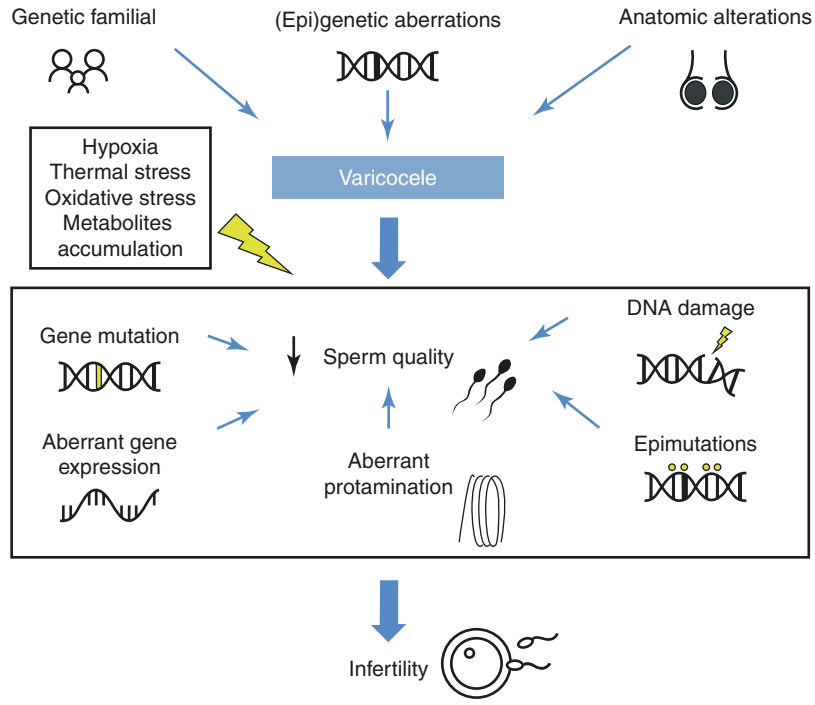

Fig. 4.1 Many factors may contribute to development of varicocele, such as genetic familial influence, (epi)genetic sporadic aberrations, and congenital anatomical malformation. The enhanced temperature, oxidative stress, hypoxia, and metabolites accumulation, aggravated by genetic and epigenetic changes, may contribute to decreased sperm quality and infertility

Somatic Chromosomal Alterations

Aneuploidies are the most common chromosomal aberrations in humans. These comprise alterations in the numbers of the chromosomes, caused by the abnormal distribution during meiosis, especially non-meiotic disjunction. The frequency of chromosomal abnormalities in male infertility is about 5% [10]. Infertile men with somatic aneuploidies generally develop spermatozoa with structural chromosomal abnormalities, and, consequently can have children with the same alteration [11]. In addition, the frequencies of autosomal and sexual chromosomal abnormalities among men with oligozoospermia and azoospermia are 4.2% and 1.5%, respectively, compared with frequencies of 0.14% and 0.25%, respectively, among normal newborn screens [12].

The incidence of chromosomal abnormalities in men with varicocele is highly variable, and it may depend on several factors, particularly for those associated with spermatic alterations. Rao et al. [13] demonstrated that the frequencies of chromosomal defects were higher (24.56%) in men with varicocele, when compared to those with idiopathic infertility (12.37%), that included inversions on chromosomes 9 and 2, translocations between chromosomes 4 and 15, deletions on chromosome 4, and insertions into chromosome 9. They also observed the addition of genetic material on the chromosomes 21 and 22. In addition, some patients who had varicocele and chromosomal defects had microdeletions in the NS153, SI158, and SI254 regions of the Y chromosome. Moreover, higher rates of severe oligozoospermia and oligoasthenoteratozoospermia were observed in patients with varicocele.

Stahl et al. [14] described the presence of a supernumerary minute ring chromosome (SMRC) with the karyotype 47,XY,+r(14)(p11.2q11.2) in an oligozoospermic patient with a varicocele. The presence of SMRC 14 seems to be related to reproductive problems [15], which may not be related to semen parameter alterations [16]. In the patient with a varicocele, it was not possible to determine whether the disorder was a phenotypic manifestation of SMRC 14 that had resulted in infertility or a coincidental finding [14].

Lee et al. [17] characterized an isodicentric Y chromosome in an azoospermic man who had a varicocele and was karyotyped as 46,X,idic(Y)(q11.22)[58]/45,X[12]. Isodicentric Y chromosomes are the most frequently observed chromosomal aberrations in humans [17]. Phenotypes of carriers of isodicentric Y chromosomes range from male to abnormal female or individual with ambiguous genitalia, according to the locations of the breakpoints and the proportions of each cell line in cases of mosaicism [18]. The patient characterized also had a breakpoint between the SY161 and SY121 regions, which resulted in the deletion of azoospermia factors (AZFs) b and c.

The Y chromosome is divided into seven deletion intervals, and each interval is subdivided into subintervals (A, B, C, etc.) (Fig. 4.2) [19, 20]. The AZF locus is mainly responsible for spermatogenesis, and it is located on the long arm of the Y chromosome at deletion intervals 5 and 6. The AZF locus has three non-overlapping subintervals, namely, AZFa, AZFb, and AZFc. Each AZF locus acts at a different stage of gametogenesis. A deletion in the AZFa region is associated with a total absence of germ cells, but with the remaining of Sertoli cells. A deletion in the AZFb

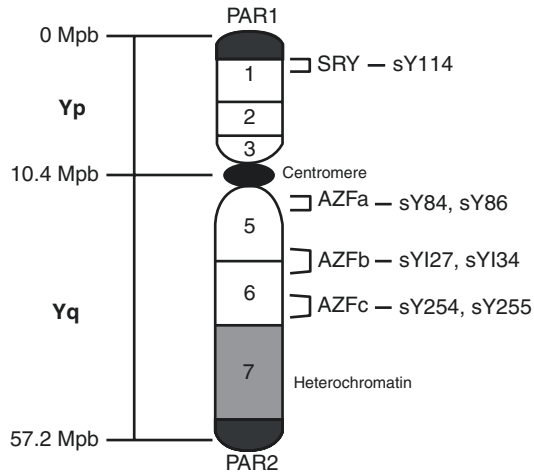

Fig. 4.2 Schematic representation of the human Y chromosome showing seven deletion intervals, three azoospermia factor regions (AZFs), and the pseudoautosomal regions (PARs), namely, PAR1 and PAR2

region is associated with the interruption of germ cell development at the pachytene stage, which causes meiotic maturation arrest. On the other hand, a deletion in the AZFc region can interrupt germ cell development, but only at the spermatid stage, which leads to low sperm counts [21].

Several studies have analyzed the relationship between deletions in the different AZFs regions and the varicocele phenotype [9, 22–26]. Moro et al. [22] found Yq deletions in patients with varicocele, all of whom had severe hypospermatogenesis (reduced number of germ line with respect to Sertoli cells), while no Yq deletions were found in the patients with mild oligozoospermia (sperm count 10–20 × 10^6/ml). Similar results have also been reported from other studies [23, 25]. In a study undertaken by Dada et al. [9], the deletion of AZFa and the partial deletion of AZFb were found in an azoospermic patient, and deletions of AZFb and AZFc were found in a severely oligozoospermic patient. de Sousa Filho et al. [26] and, more recently, Harton and Tempest [27] studied infertile patients with varicocele with phenotypes that ranged from azoospermia to mild oligozoospermia, and they found Y chromosome microdeletions only in men whose semen production was severely impaired. The testicular damage observed in patients whose semen production is severely impaired is attributed to genetic abnormalities, but it is possible that varicocele and Y chromosome microdeletions, either alone or in combination, may be the etiologic factors underlining male infertility among patients with varicocele.

Cayan et al. [27] evaluated men who underwent varicocelectomy, and showed improvements in the semen quality despite the genetic alteration. However, oligozoospermic men with varicocele may not respond to surgical correction and do not have improvements in semen parameters, when the genetic abnormality is congenital. Identifying whether the genetic alterations associated with varicocele have a somatic or a germline genetic origin is important to determine the etiology and improve treatment decision-making for these patients, which should include genetic counseling to prevent potentially poor responses to surgery.

Sperm Chromosomal Alterations

Aneuploid spermatozoa are present in the ejaculates of 3–5% of men in the general population [11]. Among infertile men, this frequency is three times higher, and is associated with the presence of different sperm-related phenotypes, including oligozoospermia, asthenozoospermia, and teratozoospermia [28]. Since the chromosomal composition of a spermatocyte influences gamete maturation and it may reduce sperm viability, high aneuploidy rates may reduce the semen quality and fertility [29]. Spermatozoa with altered morphologies and motility have high rates of chromosomal abnormalities, which cause fertilization and implantation failures, and compromise normal embryonic development [30].

A compromised testicular environment, such as in varicocele disease, may contribute to the occurrence of meiotic errors [31]. Varicocele is related to deleterious alterations at the initial stage of sperm differentiation that consequently lead to malformations of sperm acrosome and nucleus [32]. Finkelstein et al. [29] evaluated the meiotic non-disjunction rates of chromosomes 1, 15, 18, X, and Y in the sperm cells, in which the rates of disomy were 15 times higher for chromosomes 1 and 15, and seven times higher for chromosome 8 in the men with varicocele that also presented heterodisomy of the sex chromosomes. Meiotic segregation alterations were also observed by Baccetti et al. [33] who analyzed 39 men with varicoceles and found that some patients with varicocele had high rates of diploidy and disomy of the sex chromosomes.

Considering sex chromosomes, it is possible to assume that the high rate of disomy among men with varicocele results mostly from non-disjunction during the first meiotic division. These findings support the hypothesis that the damage caused by varicocele during spermatogenesis may also affect chromatid segregation. The higher non-disjunction rates in patients with varicocele may not be directly related to the disorders' phenotypes, but rather to the testicular damage caused by varicocele that leads to low semen quality, which may be related to the chromosome segregation defects.

Regarding varicocelectomy, there were no significant differences in the frequencies of chromosome 1, 16, 17, and 18 aneuploidies in the sperm cells before and 6 months after surgery [34]. However, reductions in the frequencies of chromosome 17 and 18 aneuploidies were evident after varicocele repair. Varicocelectomy may improve semen quality and slightly reduces the frequency of aneuploidies in sperm cells, especially when these alterations are in gametic cells.

Genetic Mutations and Polymorphisms

Gene variability occurs naturally and can be sporadic or inherited. These changes may have functional consequences on gene products such as non-synonymous mutations or do not modify the produced amino acid sequence (synonymous mutation) [35]. If the genetic variation occurs at a frequency of more than 1% of the population, it is called a polymorphism. These variations, such as polymorphisms and mutations, are related to male infertility, and they may directly affect spermatogenesis [36–38].

Several studies attempted to elucidate the roles that the different polymorphisms play in the infertility phenotypes among men with varicocele (Table 4.1). One of the most studied polymorphisms in men with varicocele is that found in the glutathione *S*-transferase (*GST*) gene, related with oxidative stress and antioxidant capacity [39, 41]. The *GST* genes show hereditary deletion polymorphisms that characterize the null genotypes of the *GSTM1* and *GSTT1* genes, with loss of enzymatic activity [40]. Tang et al. [48] observed that in infertile men with varicocele, the sperm concentrations and motility was worse in those with the *GSTM1*, *GSTT1*, or *GSTM1/T1* null genotypes. On the other hand, Acar et al. [40] did not observe an increased frequency of the *GSTM1* null genotype in men with varicocele. Regarding varicocelectomy, men with the *GSTT1* non-null genotype presented a better response, with higher concentrations of mobile sperm cells after surgery [49, 50].

The *Cys/Cys* null genotype of the human 8-oxoguanine DNA glycosylase 1 (*hOGG1*) gene polymorphism is present at higher frequencies in men with varicoceles [42], and 8-hydroxydeoxyguanosine is a sensitive marker of the oxidative DNA damage caused by reactive oxygen species (ROS) in human sperm cells [51]. In addition, men with varicocele and the *Cys/Cys* null genotype have the worst semen analysis results, which are not observed in patients with subclinical varicocele. The *hOGG1 Cys/Cys* genotype seems to increase the level of oxidative damage and decrease the antioxidant capacity of the seminal plasma in patients with varicocele, which may lead to a higher incidence of male subfertility [42].

Table 4.1 Genetic mutations and polymorphisms found in men with varicocele

Gene	Genotype	Consequence	Age/grade	Study
GST	*GSTM1* null	Increased susceptibility to oxidative damage	36/clinical --/I, II, III	Chen et al. (2002) [39] Acar et al. (2012) [40]
	GSTT1 null		--/-- --/I, II, III	Wu et al. (2009) [41] Acar et al. (2012) [40]
hOGG1	*Cys/Cys* null	Worst semen analysis results	21–37 (grouped)/--	Chen et al. (2018) [42]
TNP	SNP	Abnormal condensation of sperm chromatin	28/I, II, III	Heidari et al. (2014) [43]
NOS3	Lower *4b/a* and higher *G894T*	Dysregulation of endothelial NO production	28/I, II, III	Kahraman et al. (2016) [44]
MTHFR	*A1298C*	Deregulate methylation	27/I, II, III	Ucar et al. (2015) [45]
ACP1	**B/*C*	Lower sperm concentration and increased abnormal morphology	27/I, II, III	Gentile et al. (2014) [46]
P53	*Arg*/Arg**	Proapoptotic activity	33/--	Gentile et al. (2015) [47]

--Information not reported by the authors

Another mutation found in men with varicocele relates to the transition nuclear protein (*TNP*) gene. In sperm cells, histones are replaced by protamines during chromatin remodeling, because the DNA-protamine complex is more stable and less susceptible to exogenous and endogenous influences. The first step occurs in round spermatids, and it involves the replacement of histones by TNP1 and TNP2, which, in a subsequent step, are replaced by protamines [52]. Single nucleotide polymorphisms (SNPs) in the *TNP* genes are associated with DNA damage in patients with azoospermia, which causes infertility [52–54]. Heidari et al. [43] found a new base substitution in the intronic region of the *TNP1* gene in individuals with varicocele. Although these results are controversial, they suggest that this SNP may be related to infertility in these patients. Defective TNP proteins are believed to cause the abnormal condensation of sperm chromatin, increase the number of sperm DNA breaks, and to cause sperm immobility.

Low nitric oxide (NO) concentrations and high levels of nitric oxide synthase (NOS3) activity have been observed in the seminal plasma from patients with varicocele [44]. The vessel walls release NO following the *NOS3* gene expression, causing vascular relaxation. If an inadequate amount of NO is released, endothelial dysfunction and hypertension occur [55], which may contribute to varicocele onset. The *NOS3* gene has three common polymorphisms, namely, *T-786C*, *G894T*, and *4b/a*. Compared with control individuals, a lower frequency of the *4b/a* polymorphism and a higher frequency of the *G894T* polymorphism have been found in men with varicocele [44]. The *4b/a* polymorphism seems to protect against varicocele development, because this polymorphic intron regulates endothelial NO production [56]. On the other hand, the *G894T* polymorphism reduces the NO levels [57], and it may contribute to the occurrence of varicocele by preventing muscle relaxation and generating hypertension in the testicular vessels. In these cases, high levels of NOS3 activity seem to be a compensatory mechanism that protects the vascular anatomy in patients with varicocele [44].

Ucar et al. [45] showed that men who are homozygous (*1298AA*) for the methylenetetrahydrofolate reductase (*MTHFR*) gene have 2.3 times more chance to present with varicocele than men with the heterozygous (A1298C). The *MTHFR* gene encodes the enzyme involved in the conversion of homocysteine to methionine [58] *MTHFR* plays a key role in regulating the addition of methyl groups during DNA replication, which is an important mechanism underlying epigenetic regulation during spermatogenesis [59]. The most well-known *MTHFR* gene SNPs are the *C677T* and *A1298C*, which reduce enzyme activity [60], and these are associated with poor semen quality and infertility [61, 62]. Investigators have suggested that the presence of the variant *1298AA* is a risk factor for varicocele development [45].

ACP1 gene controls the activity of platelet-derived growth factor (PDGF), an important regulator male gonads development [63]. The *ACP1* polymorphic region has three codominant alleles, namely, **A*, **B*, and **C*, that generate different enzymatic activity profiles [64]. Gentile et al. [46] showed that lower sperm concentrations and increased abnormal morphology were found in men with varicocele with the genotype *ACP1 *B/*C*, which was associated with a high level of enzymatic activity. Despite the possibility that other mechanisms may be involved, the high level of PDGF activity generated by the **B/*C* genotype may have a negative effect on the fertility of men with varicocele [46].

Polymorphism in the *P53* gene is related to semen alterations in men with varicocele. The codon 72 on exon 4 encodes a proline (*Pro**), and change this amino acid by an arginine (*Arg**), affects the biochemical and functional properties of sperm [65], and induces apoptosis [66, 67]. In men with varicocele and low sperm mobility [47], the genotype *Arg*/Arg** is more frequent than *Pro*/Pro∗* genotype, that may influence the fertility of these patients [47].

Mitochondrial Genetic Modification

Mitochondria have multiple cellular functions that, in addition to their primary function of producing adenosine triphosphate (ATP), contribute to many physiological processes, including

apoptosis, calcium homeostasis, and lipid and amino acid metabolism [68, 69]. Mitochondria have their own genome, and, in mammalian species, the mitochondrial genome contains 15–17 kb of circular double-stranded DNA that includes 37 genes that encode 13 peptides, 22 transfer ribonucleic acids (tRNAs), and two ribosomal ribonucleic acids (rRNAs) [70]. Folgerø et al. [71] reported a reduction in the motility of sperm cells in individuals with structural defects in their mitochondria. More recently, the integrity and copy number of sperm mitochondrial DNA (mtDNA) have been studied in the context of male infertility [72–74]. Besides the energy generated through glycolysis, it is believed that mitochondrial ATP is required for sperm motility and hyperactivation, which suggests that mitochondrial function in sperm cells may be important for their flagellar propulsion and fertilization capacity [75, 76].

The mitochondrial genome is quite vulnerable to ROS and is thus an excellent marker of oxidative stress [77, 78]. Since mtDNA is not associated with histone proteins, it has limited damage repair mechanisms, and it is constantly exposed to the high levels of ROS generated by oxidative phosphorylation [79]. Sperm mitochondrial mutations are associated with oligoasthenozoospermia and isolated asthenozoospermia [80, 81]. In addition, the presence of mtDNA defects in the Sertoli cells and other testicular support cells may cause energy production losses and spermatogenesis failures [82].

The most frequently occurring mtDNA mutation in human sperm cells is a 4977-base pair (bp) deletion, which has been associated with reductions in fertility and sperm motility [83]. Studies' findings have shown 4977-bp deletion is frequent in men with varicocele [39, 41, 82], an important region that contains several genes important to mitochondrial respiratory chain components. Therefore its deletion can impair ATP production, alter mitochondrial respiratory functions, and cause meiotic arrest, which may lead to the formation of non-functional sperm cells [84]. These alterations increase mitochondrial ROS, a common alteration in some pathological conditions that has serial implications on the structure of mtDNA [85], such as DNA strand break and deletions [86], which could be implicated in varicocele-related infertility.

SNPs in the mitochondrial genome have been described in men with varicocele, and some are associated with low semen quality [81, 83, 87]. Heidari et al. [88] detected ten nucleotide variants in the mtDNA from infertile men with varicocele. These mtDNA variants can generate mitochondrial rearrangements, interfere with conserved codons and cause DNA strand breaks, and they can be characterized as pathogenic mutations and factors that predispose individuals to varicocele development [88].

Changes in sperm mtDNA copy number may also influence male fertility [72]. Higher numbers of mitochondria and mtDNA copies in sperm cells have been associated with defective sperm function [72, 89]. The results from Gabriel et al. [90] showed that compared with men without varicocele, the average number of sperm mtDNA copies was higher in men with varicocele and the number of mtDNA copies declined after varicocele repair. These data suggest that a high number of mtDNA copies is associated with poor sperm function, especially in relation to motility, and it may be indicative of problems relating to energy metabolism in gametes [91]. A reduction in the number of sperm mtDNA copies after varicocelectomy may be a result of improved spermiogenesis that is accompanied by an increase in the release of the residual mitochondria and the better regulation of mtDNA replication [90].

Epigenetics Mechanisms

Epigenetics involves the study of inheritable alterations (at the cellular level) that affect gene function and do not involve DNA sequence changes [92]. Epigenetic changes generally modify the structure of chromatin, leading to different gene expression programs that are specific to each cell, and they are essential for the normal development [93, 94]. Epigenetic mechanisms include histone modifications, DNA methylation, and non-coding RNA (ncRNA) expression.

Epigenetic changes, or epimutations, are associated with a variety of human diseases, including reproductive diseases and loss of fertility. Such modifications are potentially reversible and demonstrate extreme plasticity as a consequence of cellular epigenome reprogramming. Aberrant reprogramming of the epigenome during gametogenesis, such as abnormal DNA methylation and histone modification, and alterations in the ncRNAs expression, can lead to failures in the formation and maturation of sperm cells, thereby reducing their fertilization capacity and pregnancy rates, and leading to altered embryonic development or misscarriage [93, 95].

DNA Methylation

DNA methylation is the most studied epigenetic mechanism that modifies gene expression and it is generally associated with reduced gene activity or silencing [96]. In mammals, DNA methylation consists of the covalent addition of a methyl group at position 5 of the cytosine ring, resulting in the formation of 5-methylcytosine (5meC), and this mainly occurs in cytosine-phosphate-guanine dinucleotides [97]. DNA methyltransferases (DNMTs) convert cytosine to 5meC [98, 99]. The DNA may not be able to function as a substrate for DNMTs if lesions are present, which can lead to global hypomethylation and genomic instability [100]. DNA methylation is a dynamic process that occurs during human spermatogenesis [101]. Several studies have investigated the associations between methylation and semen quality and male fertility [102–105]. The DNA methylation pattern is involved in the control of the functional capacity of germ cells [106] and these alterations affect pregnancy rates [102, 107].

Bahreinian et al. [108] found lower global DNA methylation levels in the sperm cells of men with varicocele than those in fertile individuals. This study also showed that the sperm cells of the individuals with varicocele were more susceptible to DNA damage when the DNA was hypomethylated, demonstrating a negative relationship between methylation and DNA fragmentation. The same research group investigated the global sperm DNA methylation levels before and 3 months after varicocelectomy, and they found a higher rate of methylation after surgery, but the difference was not significant [109]. The subgroup analysis of the individuals in this study revealed that the improvements in DNA methylation after varicocelectomy appeared to be greater in oligozoospermic individuals who were more severely affected by varicocele.

Histone Modifications

Nuclear chromatin consists of DNA wrapped around histone proteins that package the DNA in the transcriptionally inactive heterochromatin. Histones can be modified by adding chemical groups to the tails of these proteins that form the nucleosome. The main histone modifications are phosphorylation, ubiquitination, and, in particular, methylation and acetylation that generally occur closer to the enhancers and the promoter regions [110, 111]. Regions of DNA that are tightly bound to histones are transcriptionally silenced, while weakly bound regions are transcriptionally active, directly affecting gene expression.

It was already shown that premature and/or decreased acetylation in sperm cells leads to impaired spermatogenesis, and it is associated with a reduction in protamine expression, which confirms the crucial role of histone hyperacetylation and the replacement by protamines [112]. In addition, the random retention of histones in the sperm cells of infertile men leads to changes in the methylation of the promoter regions of developmental genes, which may have a damaging cumulative effect on fertility [113]. To date, the role of histone modifications in men with varicoceles has not been evaluated. Further, it remains unclear whether varicoceles are related to protamine deficiencies [108, 109, 114, 115], and studies into the epigenetic modifications of histones are required to elucidate the sperm maturation process in men with varicocele.

Non-Coding RNAs

The mammalian genome is composed of an amount of DNA that is not translated into proteins. The non-coding RNAs (ncRNAs) are a group of versatile molecules that participates in many biological mechanisms, such as the genome regulation of gene expression. MicroRNAs (miRNAs) are a family of small ncRNAs that regulate posttranscriptional gene expression by binding to the 3′-UTR region of the target mRNA, causing mRNA degradation or translational repression [116]. They regulate up to 30% of the genes in the human genome [117] in a variety of physiological and developmental processes, including cell differentiation, proliferation, and apoptosis [118]. Several miRNAs are exclusively or preferentially expressed in the testes [119], and altered miRNA expression in sperm cells has been associated with spermatogenesis defects and reductions in semen quality [120–122].

Stress can alter the biogenesis of miRNAs and, consequently, the translation of their target mRNAs [123]. Mostafa et al. [124] analyzed the expression of miRNA-122, miRNA-181a, and miRNA-34c5 in the sperm cells of men with varicocele, and their results showed decreased levels of these miRNAs in the presence of varicocele and oligoasthenoteratozoospermia, which indicates that these miRNAs may be associated with semen quality in men with varicocele. These miRNAs were also associated with the varicocele grade and the side on which it occurred, and they were negatively correlated with oxidative stress and apoptotic markers.

miRNAs may be associated with cellular homeostasis during stress. In this context, Ji et al. [125] analyzed the expression of several miRNAs that are related to stress in sperm cells, and they found that the miR-15a expression was reduced in patients with varicocele compared with that in control individuals. miR-15a regulates the heat shock protein (HSP) A1B (*HSPA1B*) gene expression, that reduces the damage caused by hyperthermia during spermatogenesis. In an attempt to protect the cell against ROS-induced damage, the miR-15a expression declines in cells after oxidative stress [126] in order to increase the *HSPA1B* expression. These results underscore the protective role of miR-15a, which suppresses *HSPA1B* expression, and this may be one of the mechanisms that contribute to protection of hyperthermia or sperm damage induced by oxidative stress [125].

Gene Expression

Sperm cell transcripts were believed to be mRNAs that were stored after meiosis or produced during the early stages of spermatogenesis [127]. In recent years, different types of human sperm cell RNAs have been described, as well as the demonstration of some translation activities that give rise to proteins possibly involved in sperm quality and embryonic development [128]. The function of most sperm cell transcripts remains unknown, but different RNA populations are strongly associated with sperm motility and function and other semen parameters [129]. The sperm cell gene expression may have important clinical implications for the diagnosis of male infertility and for assisted reproduction techniques [130].

Varicocele may alter the expression profiles of several genes, since its presence causes thermal and oxidative stress that may compromise DNA and RNA integrity and the function of the Sertoli cells [5]. Some studies have investigated the relationships between varicocele and the expression of genes that are fundamental to spermatogenesis and sperm maturation (Table 4.2). For example, Zalata et al. [131] observed that the androgen receptor *(AR)* gene expression was lower in infertile men with varicocele than that in fertile control individuals. The *AR* gene expression is required for sexual differentiation, regulation of spermatogenesis, completion of meiosis, and transition from spermatocytes to spermatids [140].

Alterations in the process and the rate of cell death can also reduce the semen quality. Del Giudice et al. [132] observed higher levels of mRNA expression for the Fas protein ligand (FasL), which is an important apoptotic marker, in adolescents with varicocele. Mostafa et al.

Table 4.2 Alterations in genes expression reported in men with varicocele

Gene	Alteration	Age/grade	Study
AR	Downregulated	-----	Zalata et al. (2013) [131]
FasL	Upregulated	16/II, III	Del Giudice et al. (2010) [132]
BAX	Upregulated	31/I, II, III	Mostafa et al. (2014) [133]
BCL2	Downregulated	31/I, II, III	Mostafa et al. (2014) [133]
HSPA4, HSF1, and *HSF2*	Upregulated	30/II, III	Ferlin et al. (2010) [134]
HSPA2	Downregulated	--/I, II, III 18/II, III	Yeşilli et al. (2005) [135] Lima et al. (2006) [136]
PLCζ	Downregulated	--/II, III	Janghorban-Laricheh et al. (2016) [137]
MT1M and *PHLDA1*	Downregulated (after varicocelectomy)	24/III	Oliveira et al. (2012) [138]
CCIN and *PRM2*	Upregulated (after varicocelectomy)	24/III	Oliveira et al. (2012) [138]
Ropporina	Upregulated (after varicocelectomy)	32/I, II, III	Amer et al. (2015) [139]

--Information not reported by the authors

[133] reported reduction in the semen B-cell lymphoma-2 (*BCL2*) gene expression and an increase in the BCL2-associated X protein (*BAX*) gene expression in men with varicocele. Members of the BCL2 family of proteins are involved in regulating apoptosis in several cell types and they can inhibit (BCL2) or promote apoptosis (BAX) [141]. Supporting the hypothesis that apoptotic factors influence semen quality, increased expression of *FASL* [132] and *BAX* [133] were associated with lower sperm concentration, motility and morphology, while *BCL2* showed a positive correlation with these parameters [133]. Changes in the expression of genes related to cell death in men with varicocele exacerbate the deterioration of the semen parameters.

Several mechanisms may cause germ cell apoptosis that is induced by thermal stress [142], and HSPs are active to enable cells to survive lethal thermal stress conditions [143]. The sperm cells from oligozoospermic men with varicocele showed increases in expression of the *HSPA4*, heat shock factor (*HSF*) 1, and *HSF2* [134], while *HSPA2* expression was downregulated [135, 136]. Differential expression of these mRNAs was associated with spermatogenic damage in varicocele, thus representing markers of cellular response to the spermatogenic thermal stress induced by the disease.

Regarding fertility, men with varicocele have lower expression of the phospholipase C ζ (*PLCζ*) gene than that in fertile men who do not have varicocele [137]. The PLCζ protein is an important oocyte activator, and the differential expression of this gene has been associated with impaired fertilization [144, 145]. Sperm cells that have defects in their abilities to activate the oocyte have a lower chance of fertilization, even if they can penetrate the oocyte [137].

Changes in the levels of the expression of several genes have been demonstrated before and after varicocelectomy [115, 138, 139]. Oliveira et al. [138] showed reductions in the expression of *MT1M*, which helps to protect against oxidative damage, and *PHLDA1*, which is a mediator of apoptosis, and increases in the expression of *CCIN*, which encodes a protein that contributes to the preservation of the integrity of sperm nuclei, and *PRM2*, which is associated with DNA condensation. Amer et al. [139] demonstrated an upregulation of the gene that encodes ropporin, which is a protein component of the flagella's fibrous sheath, and this correlated with gamete motility after varicocele correction. Ni et al. [115] demonstrated that the protamine-1/protamine-2 mRNA ratio returned to normal after varicocele correction, and the patients who underwent surgery had lower DNA fragmentation indexes and higher pregnancy rates. These differences in gene expression before and after varicocelectomy show that testicular function recovers after surgery, and this

Table 4.3 Genetic and epigenetic alterations described in men with varicocele and the resulting phenotype associated

Alteration	Consequence	Phenotype
Chromosomal abnormalities	Gain or loss of chromosomal segments	Oligoasthenoteratozoospermia and azoospermia
Genetic mutations and polymorphisms	Genetic dysregulation	Low sperm count and quality, oxidative damage and DNA fragmentation
Changes in mtDNA structure and number of copies	Deregulation of gene expression and DNA damage	Poor sperm function
Loss of global DNA methylation	Genomic instability and DNA damage	Susceptible to DNA damage
Decreased levels of miRNAs	Deregulation of gene expression	Oligoasthenoteratozoospermia and oxidative damage

is demonstrated by improvements in the semen parameters, and, consequently, the fertility of these patients.

Conclusions and Perspectives

Genetic and epigenetic factors are associated with poor sperm quality and production in men with varicocele, and consequently, the loss of fertility, regardless of the degree of disease. The variety of phenotypes observed suggests a multifactorial condition, in which the anatomic alterations related can lead to modifications in the testicular environment that may affect spermatogenesis. As a well-orchestrated process, any failure can impair sperm production and reproductive potential. Varicocelectomy is the main treatment strategy to improve seminal quality, nevertheless little is known regarding the genetic and epigenetic modifications associated with treatment outcomes. Due to the multifactorial and heterogeneous nature of varicocele, no specific biomarkers could be identified; however, several alterations, such as chromosomal aberrations, gene polymorphisms, mutations, SNPs, changes in mtDNA, and epigenetic mutations, are frequently observed in this condition (Table 4.3). However, the identification of the genetic and epigenetic alterations related to and the main mechanisms involved in varicocele will improve understanding of the disease. It may help to prevent varicocele, and guide patients' treatment and prognosis, especially for those with infertility.

Review Criteria

We used the search function of the US National Library of Medicine, National Institutes of Health, that is, PubMed, which is available at the National Center for Biotechnology Information, to find studies that correlated the presence of varicoceles with genetic disorders. We identified studies and extracted data by using the following keywords: varicocele and chromosome, aneuploidy, aneusomy, centromere, mitochondria, DNA, RNA, gene, genetic, polymorphism, mutation, genome, transcriptome, epigenetics, methylation, chromatin, histone, and telomere. Only articles published in English until March 20, 2018, were considered. Data published exclusively for conferences, websites, or books were not included.

Multiple Choice Questions and Answers

1. Regarding the genetic and epigenetic basis and the alterations that underlie the variable reproductive potential of men with varicocele, which of the following statements is incorrect?
   (a) Genetic abnormalities contribute to spermatogenesis failure and loss of fertility in varicocele.
   (b) Deletions in the mitochondrial DNA (mtDNA) of spermatozoa have been reported in men with varicocele.

(c) Abnormal sperm morphology and motility are related to higher rates of chromosomal abnormalities, gene mutations, polymorphisms, and epigenetic alterations.
(d) **Varicoceles have an inheritable genetic component that is the major cause of infertility.**
(e) Epigenetic modifications are reversible and have great plasticity, being reprogrammed during gametogenesis.

2. Sperm chromosomal alterations are the major cause of male infertility which mostly originate from:
(a) Abnormal mitotic segregation.
(b) **Abnormal meiotic segregation.**
(c) DNA damage.
(d) Loss of DNA methylation.
(e) Gene mutation.

3. Mutations may cause variabilities in the DNA sequences and:
(a) Do not alter the function of the genes because they are known polymorphisms.
(b) Affect the sperm function in men with varicocele, but without alteration of genes expression related to infertility.
(c) **Lead to altered gene function and consequently poor seminal quality and infertility in men with varicocele.**
(d) Are always spontaneously corrected by the oocyte at the time of fertilization, preventing changes in embryonic gene expression.
(e) Affect important genes for seminal quality in men with varicocele, except polymorphisms that do not alter gene expression.

4. Increased temperature, hypoxia, oxidative stress, and toxic metabolite accumulation found in the testicle with varicocele may lead to:
(a) Poor semen quality and fertility without any genetic or epigenetic alteration in the spermatozoa.
(b) DNA damage, genomic instability, and gain of global DNA methylation in the spermatozoa.
(c) Aberrant epigenetic alterations that cannot be reprogrammed.
(d) **Genomic instability, DNA damage, aberrant epigenetic alterations in the spermatozoa.**
(e) None of these answers is correct.

5. Environmental factors in the testicle may modulate the expression of important genes related to spermatogenesis by epigenetic changes on DNA. What are the main recognized epigenetic mechanisms that control gene expression?
(a) DNA methylation, histone modification, and chromatin modulation.
(b) DNA methylation, histone modification, and telomeres.
(c) Histone modification, ncRNAs, and telomeres.
(d) **DNA methylation, histone modification, and ncRNAs.**
(e) Histone modification, telomeres, and chromatin modulation.

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5 Oxidative Stress and Varicocele Pathophysiology

Ahmad Majzoub, Chak-Lam Cho, Ashok Agarwal, and Sandro C. Esteves

Key Points

- Oxidative stress (OS) occurs when the redox equilibrium is not maintained due to imbalance between reactive oxygen species and their neutralizing antioxidants.
- The testicular and epididymal responses to hyperthermia, hypoxia, and reflux of toxic metabolites can cause a state of OS and, consequently, influence sperm quantity and quality.
- Direct and indirect measures of OS have been found to be elevated in patients with varicocele.
- Varicocele repair was associated with significant reductions in OS measures.
- Antioxidant therapy either alone or with varicocele repair may exert beneficial effects on OS measures, though long-term effects still require validation.

Introduction

Approximately 15–20% of adult men may have a varicocele, though the condition is more prevalent in infertile men (40%) [1, 2]. Varicocele was suggested to induce male infertility through several pathophysiologic mechanisms that can impair spermatogenesis. Proposed potential mediators of varicocele's effect on male reproduction include elevated scrotal temperature, reflux of toxic metabolites into the internal spermatic vein, hypoxia, antisperm antibodies, hormonal dysfunction, and oxidative stress (OS) [3]. The latter occurs when the redox equilibrium is not maintained due to imbalance between reactive oxygen species (ROS) and their neutralizing antioxidants.

ROS are highly reactive substances that can exert several physiological functions via interactions with different biological molecules (amino acids, lipids, and nucleic acids) [4]. Immature sperm cells and leukocytes are believed to be the primary sources of seminal ROS [5, 6]. To a lesser extent, ROS can be generated in semen by epithelial cells [7]. ROS are

A. Majzoub (✉)
Department of Urology, Hamad Medical Corporation, Weill Cornell Medicine Qatar, Doha, Qatar

C.-L. Cho
S.H. Ho Urology Centre, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

A. Agarwal
American Center for Reproductive Medicine, Department of Urology, Cleveland Clinic, Cleveland, OH, USA

S. C. Esteves
ANDROFERT, Andrology & Human Reproduction Clinic, Campinas, SP, Brazil

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vital products for optimal sperm function through regulating capacitation processes—hyperactivation and acrosomal reaction—and, consequently, fertilization.

Despite such important physiologic effects, excessive amounts of ROS can be harmful, as they can exacerbate sperm DNA damage, lipid peroxidation, and abortive apoptosis [8]. Antioxidants scavenge the unpaired valence electrons of ROS and, therefore, control their oxidizing chemical reactions and prevent such deleterious adverse effects from happening.

Since OS markers were detected in serum, semen, and testicular samples from varicocele patients, it was suggested that OS may have a central role in the pathogenesis of varicocele-induced infertility [9–12]. However, these mechanisms have not yet been fully described and require further clarification. It was hypothesized that the testis of varicocele patients can react to stressors, such as hyperthermia or ischemia with compensatory mechanisms that generate excessive ROS [13]. These responses can exacerbate OS and consequently cause infertility. ROS target cell membranes, where they interact typically with the electron-rich polyunsaturated fatty acids (PFAs) to produce malondialdehyde (MDA). PFAs are abundant in the plasma membrane of sperm, and as such, sperm are more vulnerable to oxidative damage [14]. MDA is usually measured to assess the extent of lipid peroxidation which results in sperm membrane structural deterioration, thereby altering membrane fluidity and leading to defects in sperm motility and fertilization [15]. Excessive ROS can induce apoptosis in mature spermatozoa [16]. The apoptotic machinery comprises a family of proteases termed caspases [17], where caspase 3 is considered to have the best correlation with apoptosis, among other identified caspases [18]. In addition, ROS can alter DNA molecules eliciting single- or double-stranded DNA breaks, base shifts, and point mutations influencing sperm DNA function and possibly affecting fertilization [19]. This chapter aims to review various varicocele pathophysiologies, in which OS plays a central role in. Further, we report the clinical evidence linking OS with varicocele.

Mechanisms of Oxidative Stress in Varicocele

The pathophysiology of varicocele is multifactorial, with no single conclusive theory that can explain the controversies and discrepancies surrounding varicocele pathology. OS may be the common intermediary factor for the testicular dysfunction seen in varicocele. That being said, OS was also observed in fertile men with varicocele, suggesting that other influences may coexist to exacerbate or alleviate the effects of OS on fertility potential in men with varicocele. In a varicocele environment, the following pathophysiologic responses have been detected.

Testicular Response to Scrotal Hyperthermia

Its location within the scrotum in addition to the countercurrent heat exchange mechanism supported by the pampiniform plexus of veins allows the testicle to have a temperature that is about 2.2 °C lower than the intra-abdominal temperature. This temperature gradient, which is necessary for optimal spermatogenesis [20], may be obliterated in varicocele patients as increases in scrotal temperature by 2.6 °C have been reported [21]. Nevertheless, scrotal temperatures varied between fertile and infertile men with varicocele [22], suggesting that testicular hyperthermia cannot solely explain varicocele-related infertility.

ROS is produced in varicocele patients under heat stress or hypoxic conditions from three components including the principal cells in the epididymis, the endothelial cells in the dilated pampiniform plexus, and the testicular cells (developing germ cells, Leydig cells, macrophages, and peritubular cells) (Fig. 5.1).

NO can be synthesized by three different nitric oxide synthases (NOS): inducible NOS (iNOS), constitutive neuronal NOS (nNOS), and constitutive endothelial NOS (eNOS). Several studies have assessed the expression of these NOS in varicocele patients. A study [23] measured the iNOS and eNOS expression levels in adolescents with left-sided varicocele and reported higher

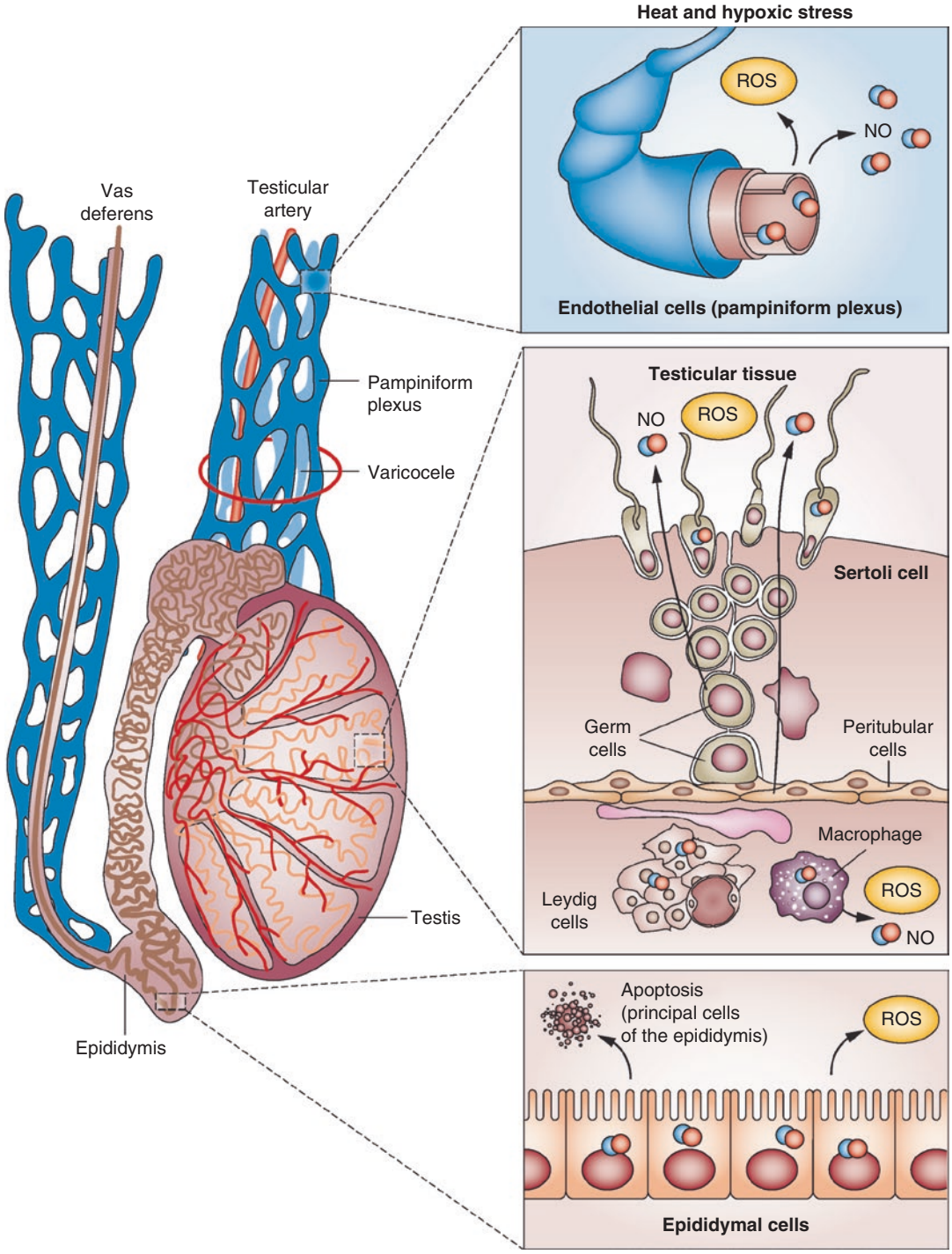


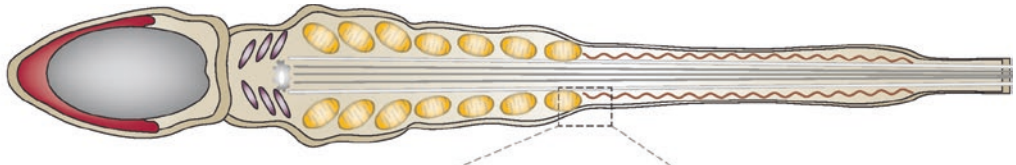
Fig. 5.1 Reactive oxygen and nitrogen species generation in infertile men with varicocele. Three components can release ROS in men with varicocele under heat and hypoxic stress: the principal cells in the epididymis, the endothelial cells in the dilated pampiniform plexus, and the testicular cells (developing germ cells, Leydig cells, macrophages, and peritubular cells). Abbreviation: ROS, reactive oxygen species; NO, nitric oxide. (Reprinted from Agarwal et al. [13]. With permission from Springer Nature.)

iNOS expression in Leydig cells compared to controls with no difference in the expression of eNOS between the two groups. Similarly, another study [24] demonstrated that men with grades II–III varicocele had higher NO levels and iNOS expression in intratesticular fluid and testicular tissue, respectively, in comparison to men with grade I varicocele or healthy controls, with no differences observed in eNOS or nNOS expression. These studies suggest that iNOS may be the main NO source in varicocele patients. iNOS is mainly expressed in peritubular testicular macrophages, with less expression in Sertoli, Leydig, and germ cells [25]. Heat stress can induce the upregulation of iNOS, with consequent increase in NO generation that deteriorate the balance towards OS [26]. High levels of NO can interfere with complexes I and IV of the respiratory chain, inhibiting ATP production [27, 28] and promoting the release of excessive amounts of superoxide free radicals by complex III (Fig. 5.2). Excessive NO can further exacerbate OS by inhibiting glutathione reductase and as a result reduced antioxidant glutathione [29]. To confirm the role of NO in varicocele-induced apoptosis and impaired spermatogenesis, experimental interventions were done using NOS inhibitors, such as aminoguanidine and Nω-nitrol-arginine methyl ester (l-NAME), in experimental rat models of varicocele. NOS inhibitor utilization decreased apoptosis and sperm DNA fragmentation and improved testicular spermatogenesis and semen parameters [30–34]. Moreover, a study in which NOS was knocked out in experimental mice confirmed better protection against heat-induced apoptosis with higher testicular weight and sperm parameters [35].

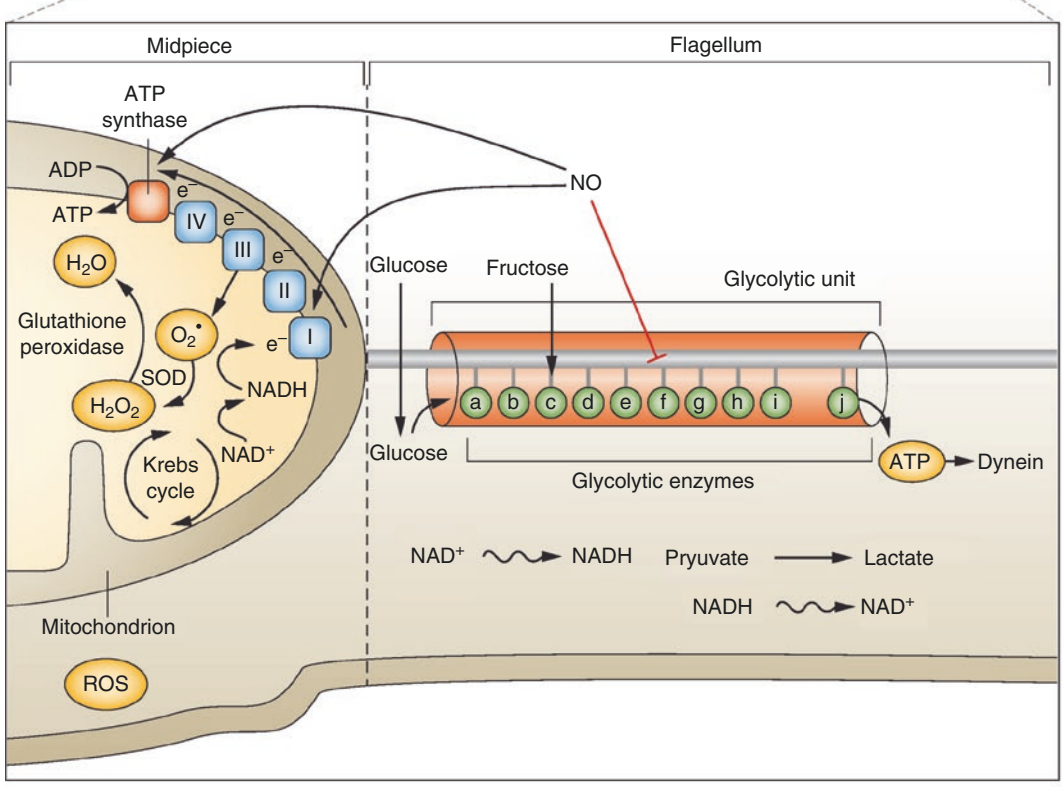
Heat exposure can elevate mitochondrial ROS production. Three mechanisms were proposed to explain the effects of hyperthermia on mitochondrial ROS generation: (1) directly inhibit mitochondrial complexes I and VI, altering the regular flux of electrons through the respiratory chain and inhibiting ATP synthesis [36], (2) accelerate cellular metabolism and energy expenditure resulting in mitochondrial ROS production, and (3) heat-induced increase in metabolic activity is not accompanied by increased blood flow, which results in hypoxia that is responsible for mitochondrial ROS generation [37]. Since, under heat stress, a lower sperm mitochondrial respiratory activity was observed [38], the first mechanism was embraced, whereas the second and third theories were refuted. Mitochondrial uncoupling proteins (known as UCPs) have been found to be downregulated in response to heat-stress-induced OS [39]. These proteins, which are located along the inner mitochondrial membrane, relocate protons from the intermembrane space to the matrix, thereby reducing the availability of protons to stimulate ATP synthesis and, consequently, decreases ROS production. UCP2 was downregulated along with an increased ROS production in chicken muscle cells exposed to acute heat [40]. However, the protective role of UCP, especially UCP2, against ROS production or OS in men with varicocele has not been studied yet. Interestingly, UCP2 needs cofactor co-enzyme Q, which is known to be reduced in men with varicocele [41], indicating that the protective effect of UCP2 can be seriously compromised.

Xanthine dehydrogenase is an enzyme that converts xanthine to hypoxanthine and uric acid, while xanthine oxidase converts xanthine to superoxide and H2O2. Under heat stress and hypoxic conditions, the oxidase form predominates [42, 43] converting heat-induced metabolites such as adenosine, inosine, xanthine, and hypoxanthine, to water-soluble products emitting ROS in the process. This enzyme is highly expressed in the post-spermatid stages [44, 45]. A sevenfold increase in the xanthine oxidase activity was demonstrated in the endothelial cells of varicocele patients [46].

Heme oxygenase (HO) is an antioxidant enzyme present in the endoplasmic reticulum, mitochondria, and plasma and nuclear membranes. It exists in two forms; inducible HO-1 and constitutive HO-2. In the human testis, HO1 has been detected in Sertoli and Leydig cells, interstitial macrophages, and some germ cells with marked elevation in Leydig cells observed under stressful conditions [47]. However, HO-2 is primarily present in maturing germ cells, spermatocytes, round spermatids, and residual bodies [48]. HO-1 antioxidant activity is achieved by



1. Heat stress inactivates mitochondrial complexes I and IV, and promotes complex III to generate excessive ROS
2. Hypoxia promotes mitochondrial complex III to release ROS
3. NO-mediated S-nitrosylation of complexes I and IV promotes execessive generation of ROS by complex III



a Hexokinase
b Glucose-6-phosphate isomerase
c Phosphofructokinase
d Aldolase
e Triosephosphate isomerase
f Glyceraldehyde-3-phosphate dehydrogenase
g Phosphogluconate kinase
h Phosphoglycerate mutase 2
i Pyruvate kinase
j L-Lactate dehydrogenase

Fig. 5.2 Varicocele-induced sperm biochemical pathways of ROS generation. In the mitochondria, heat and hypoxic stress can directly activate complex III of the electron transport chain to release ROS. NO, generated from testicular and endothelial cells in the testis with varicocele, can nitrosylate complexes I and IV to promote excessive release of ROS by complex III. In the sperm tail, where glycolytic units are present, NO can nitrosylate glyceraldehyde-3-phosphate dehydrogenase, contributing to intracellular acidification through reducing the ratio of NADH to NAD+ and reducing the production of lactate. Abbreviations: ROS, reactive oxygen species; SOD, superoxide dismutase. (Reprinted from Agarwal et al. [13]. With permission from Springer Nature.)

degradation of heme to biliverdin and bilirubin which are powerful ROS-scavenging agents [49]. Heat stress can suppress the expression of HO-1 in many human cell lines [50–52]. Studies comparing varicocele patients to healthy men have reported significantly lower seminal levels of HO-1 and bilirubin in the former compared to the latter [53], while significantly higher testicular tissue-Leydig cell reactivity to HO-1 were observed in varicocele patients [54]. This improved reactivity may help protect the Leydig cells against the damaging effects of OS [54]. Variations in HO-1 activity among men with varicocele may help explain why some have normal fertility potential while others are infertile.

Heat shock proteins (HSPs) are constitutive cellular proteins involved in the regulation of various cellular pathways including transport, transcription, translation, and signal transduction [55]. These proteins are activated in response to heat exposure, hypoxia, and OS to correct any protein misfolding that may aggravate apoptosis. HSPs are insufficiently produced in varicocele men, rendering them more prone to apoptosis and infertility. Various studies have confirmed the presence of significantly lower levels of HSPA2, mRNA, and protein expression in oligozoospermic men with varicocele compared with healthy men and normozoospermic men with varicocele [56–58]. Furthermore, varicocelectomy improved protein activities of protective HSPA2 [56]. In addition, a study reported an elevation in heat shock factor 1 (HSF 1) in oligozoospermic varicocele patients [59]. HSF1 controls HSPs expression and is known to induce apoptosis [60]. Still, new studies are needed to explore the mechanisms influencing HSP expression in germ cells, as they may differ from those observed in somatic cells.

Testicular Response to Hypoxia

Ischemia was suggested as a possible mechanism for infertility in varicocele patients as testicular tissue samples have shown germ cell disintegration, Leydig cell atrophy, and tubular basement membrane fibrosis accompanying arteriolar obstruction by microthrombi [61].

Hypoxia activates and stabilizes the hypoxia-inducible factor (HIF)1 which can induce angiogenesis and glycolysis. ROS were shown to aggravate the levels HIF1α as detected from samples taken from the internal spermatic veins of infertile varicocele patients [62]. The mechanism by which ROS can activate and stabilize HIF-1α has not been clarified yet; however, it was proposed that ROS might stimulate certain genes responsible for HIF1α production and at the same time inhibit its degradation [63]. Moreover, HIF1α can trigger eNOS to increase NO levels, resulting in vasodilation of testicular microcirculation [64, 65]. In addition, hypoxia can increase ROS production from the mitochondrial respiratory chain (complex III) and through enhancing the activity of enzymes such as xanthine oxidase, nicotinamide adenine dinucleotide phosphate oxidase 5 (NOX5), and phospholipase A2 [66].

Glycolysis products lactate and pyruvate are believed to be excessively produced in response to hypoxia [67]. However, inconsistent and conflicting results were reported [68]. While spermatic vein samples from varicocele patients were not found to contain significantly higher pyruvate and lactate levels compared to samples obtained from men with obstructive azoospermia in one study [69], reduced lactate and pyruvate levels were reported by another study [70], along with elevated lactate dehydrogenase activity in men with varicocele [58]. This can be explained by the effects of NO on glycolysis cascades occurring in the sperm tail. NO can inhibit nitrosylate glyceraldehyde3-phosphate dehydrogenase, thereby decreasing intracellular PH (through altering the NADH:NAD+ ratio) and inhibiting lactate production (Fig. 5.2) [71]. In addition, hypoxia can suppress the expression of aquaporin9, which is the main lactate influx channel between Sertoli cells and the developing germ cells resulting in such biochemical changes [72].

Hypoxia can increase the expression of leptin hormone whose receptors are expressed on sperm and Leydig cells [73]. Higher levels of leptin were expressed in germ cells of oligozoospermic varicocele men as compared with fertile ones [73]. This increased expression of leptin in

varicocele patients was considered a compensatory mechanism to protect spermatogenesis [73]. On the other hand, the increased production of leptin in germinal and Leydig cells of varicocele patients may also indicate a possible relationship between leptin and OS; however, such an assumption has not been confirmed yet. Leptin can increase lipid peroxidation and mitochondrial superoxide anion generation in endothelial cells of obese patients, suggesting a negative regulatory effect on male fertility in obese men [74, 75]. Hence, further studies are needed to elucidate leptin's role in the pathophysiology of infertility in varicocele patients.

Finally, hypoxia can increase the testicular levels of interleukin (IL)1 and IL-6, which are known inducers of ROS production in various tissues [76–78]. IL-1 and 6 are pro-inflammatory cytokines that can increase leukocyte counts in seminal fluid. Leukocytospermia is considered to be a major source of ROS, as seen in seminal studies from infertile men with varicocele [79].

Testicular Response to Reflux of Metabolites

Venous reflux is the principal finding of Doppler or venographic studies that confirms the diagnosis of varicocele. This finding may hence suggest that toxic metabolites from renal or adrenal origin can reach the endothelial cells of the internal spermatic vein and/or the testicular tissue. Compounds such as urea, prostaglandin (PG) E, PGF2α, and noradrenaline [80, 81] are known to induce cellular OS in several tissues. Excessive amounts of urea can alter glutathione levels through its detrimental effects on protein structure and function through aggravated carbamylation, a process that results from the interaction between isocyanic acid and certain free protein functional groups [82]. PGE and PGF2α have opposing effects; while PGF2α can increase ROS production, PGE decreases ROS production, and its levels are elevated in response to OS induced by PGF2α. Noradrenaline is a vasoconstrictor, inducing hypoxia and triggering the release of ROS.

Testicular Response by Cadmium Accumulation

Cadmium, from industrial sources or cigarette smoke, can be absorbed into the body by direct ingestion and inhalation or through cutaneous absorption. Varicocele patients were reported to have higher levels of cadmium in samples obtained from their spermatic veins, testicular tissue, and semen [83–85]. Regardless of the smoking status, cadmium can build up in varicocele patients through the blood-testis barrier, which is rendered porous due to elevation in hydrostatic pressure and hypoxia [85]. However, the mechanism of such build-up of cadmium is not fully understood.

Cadmium is a prooxidant that exerts a negative effect on spermatogenesis. This effect is believed to result from increased production of hydroxyl radical, superoxide anion, H2O2, or NO [85]. Cadmium, as well, can reduce the antioxidant zinc concentration, resulting in increased sensitivity to OS [83, 85]. However, the process by which cadmium can induce lipid peroxidation, whether a direct effect of cadmium, an imbalance of redox potential, or a decrease in glutathione content, is not clear.

Epididymal Response

The epididymis which plays a central role in sperm maturation and transport has several epididymal tubule principal cell linings that are capable of generating ROS [86]. In addition to the metabolically active principal cells, other sources of epididymal ROS include the luminal fluid spermiated from the testis and the endothelial cells in the rich capillary network around the caput [86, 87]. The redox balance within the epididymis is maintained by the counteracting enzymatic and nonenzymatic antioxidants. Stressful conditions such as hypoxia and heat stress can trigger overproduction of ROS by the principal cells along with compromised antioxidant production in the epididymal tubules, resulting in OS environment within the epididymis [88].

An experimental animal model, in which varicocele was induced, resulted in increased apoptosis of principal epididymal cells and decreased carnitine (antioxidant) levels, and α-glucosidase activity [89, 90]. Epididymal carnitine and α-glucosidase activity reflect the functional status of the epididymis highlighting its involvement in the defective sperm maturation often seen in infertile men with varicocele.

Testicular Response by Adrenomedullin

Adrenomedullin is a peptide hormone with vasodilator properties synthesized and secreted by several cells such as the adrenal medullary cells, testicular Sertoli and Leydig cells, germ cells, and vascular endothelial and smooth muscle cells [91–93]. Hypoxia, in addition to other cytokines and hormones, can stimulate adrenomedullin secretion [94, 95]. Inconsistent data were reported regarding adrenomedullin effects; while some have reported protective antioxidant-like effects on sperm motility [96], others have suggested that adrenomedullin may have negative effects on enzymatic antioxidants such as superoxide dismutase [97]. Moreover, adrenomedullin can indirectly activate iNOS and increase NO production, and inhibits Leydig-cell steroidogenesis [98, 99].

Infertile varicocele patients were reported to have higher levels of adrenomedullin in their internal spermatic veins as compared to peripheral veins [100]. This finding was hypothesized to result from increased secretion of adrenomedullin in response to hypoxia [101]. However, further research is needed to clarify the role of adrenomedullin in varicocele patients.

Clinical Assessment of OS in Varicocele

OS is thought to be the main mechanism of varicocele-induced impaired spermatogenesis. Several studies have confirmed the presence of higher levels of OS markers in semen samples of infertile varicocele patients in comparison to fertile men and infertile men without varicocele [102–106]. Elevated ROS level is a common finding in varicocele patients with abnormal sperm parameters. However, it is also observed in varicocele patients with normal sperm parameters, suggesting that better antioxidant defense mechanisms in those patients may explain this discrepancy.

OS Markers in Varicocele

Direct and indirect methods were established to assess OS. While direct OS measurements include total or specific ROS level evaluation in semen and total antioxidant capacity (TAC), indirect measurements include the assessment of lipid peroxidation products such as malondialdehyde (MDA) or hexanoyl-lysine, protein oxidation products such as protein carbonyl, and oxidized DNA such as 8-hydroxy-2′-deoxyguanosine (8-OHdG). Chemiluminescence can measure both the intracellular and extracellular ROS levels, whereas MDA can be easily measured by the thiobarbituric acid reactive substances (TBARS) assay [107].

Several studies have assessed direct OS markers in infertile varicocele patients reporting higher ROS levels in comparison to fertile men with varicocele [102–106]. Higher seminal levels of NO, NOS, H2O2, and superoxide anion were also observed in semen samples of infertile varicocele patients as compared to fertile healthy men [23, 46, 108–111].

Direct assessment of OS markers includes, also, seminal evaluation of enzymatic and nonenzymatic antioxidant levels, whether specifically or as TAC. One meta-analysis has confirmed the presence of significantly lower TAC levels in infertile varicocele patients in comparison to controls [112]. The ROS-TAC score, which better reflects fertility potential [113], is significantly lower in semen samples obtained from normozoospermic varicocele patients in comparison to fertile men [114]. Specific measurements of catalase and glutathione peroxidase have revealed significantly lower levels in infertile varicocele men [104, 111]. However, assessment

of seminal SOD yielded conflicting results. SOD can be either unchanged [115], increased, or decreased [104, 111].

With regard to indirect OS markers, high levels of MDA were detected in infertile varicocele patients. El Kamshoushi et al. compared testicular tissue MDA and caspase 3 levels between two infertile groups: nonobstructive azoospermia patients with varicocele and obstructive azoospermia patients. The authors reported significantly higher MDA and caspase 3 levels in the varicocele group compared with the obstructive azoospermia group. Furthermore, a direct relationship was observed between MDA, caspase 3 levels, and varicocele grade [116]. A statistically positive correlation between testicular caspase 3 and MDA levels was also reported in the varicocele group, highlighting the role of ROS and regulators of apoptosis in the pathophysiology of infertility in patients with varicocele-associated azoospermia. Animal studies have also confirmed the presence of high MDA levels in testes of rats, following experimental varicocele induction [117]. Contrary to these results, Koksal et al. [118] failed to find a statistically significant difference in MDA levels between two infertile groups, with and without varicocele. This finding, however, could be attributed to the choice of the control group which included patients with impaired spermatogenesis. Other indirect markers, such as seminal hexanoyl-lysine and 8-OHdG, were also elevated among infertile men with varicocele [109].

Chen et al. [119] measured the apoptotic index (AI) in 30 varicocele patients and 15 fertile controls, which was significantly higher among varicocele patients. Saleh et al. [120] assessed apoptosis by measuring apoptotic DNA damage due to activation of caspase-activated DNase. The study confirmed the direct effect of ROS on apoptotic sperm DNA damage which were significantly elevated in infertile varicocele patients.

Varicocele patients have higher ROS levels that correlate with the extent of sperm DNA fragmentation [119, 121]. Infertile varicocele patients were found to have a higher percentage of sperm nuclear DNA damage than infertile patients with no varicocele, irrespective of their semen parameter result [122–124]. Spermatic veins play an important role in the pathogenesis of varicocele-induced infertility, where endothelial cells can generate excessive amounts of ROS in response to various stresses. Plasma levels of OS markers in samples obtained from the spermatic and peripheral veins have revealed significantly higher NO, iNOS, xanthine dehydrogenase/oxidase, MDA, H2O2, and protein carbonyl content in infertile varicocele patients compared with controls [125–127].

Impact of Varicocele Repair on OS Markers

Varicocele repair in infertile men has been associated with significant improvements in various biomarkers of male infertility, such as semen parameters and pregnancy rates [128]. Varicocelectomy decreases or at least normalizes OS markers such as 8-OHdG [109, 129], MDA [10], nitrate, and nitrite content [109] which are known to be elevated in sperm cells of infertile varicocele patients. Moreover, varicocele repair was associated with improvements or normalization of the seminal and peripheral blood plasma TAC [130] and seminal antioxidants including α-tocopherol [10] ascorbate [10, 129], retinol, selenium, and zinc [10].

However, some studies have failed to show any beneficial effect for varicocele repair in alleviating OS markers. A study has demonstrated no difference in germinal lactate dehydrogenase (LDH) and MDA levels following varicocelectomy [131]. Also, in another study, there was no change in NO concentrations after varicocele repair [132]. This can be attributed to the study group that included younger patients without a prior history of infertility, and hence, might have enhanced protective mechanisms against OS. In addition, varicocelectomy did not reduce seminal MDA levels of adolescents with varicocele in a study by Lacerda et al. despite revealing improvements in sperm DNA integrity and mitochondrial activity postoperatively [133]. Failure of varicocelectomy to influence the levels of OS markers in this study might be due to

non-elevated preoperative OS levels in the adolescents group. However, it is unclear whether varicocele, over time, can affect OS levels in adolescents.

The time required for the beneficial effects of varicocele repair on OS markers is variable and inconsistent among studies. The benefits appear to be proportional to the postoperative duration, with significant ROS decline occurring as early as 1 month following surgery [134]. On the other hand, sperm DNA fragmentation appears to require more time for improvement with an average of 6 months [134]. One study reported reductions in ROS including NO, H2O2, and MDA and elevations of antioxidants, including SOD, catalase, glutathione peroxidase, and vitamin C at 3 and 6 months following varicocelectomy [10]. Furthermore, 6 months after varicocele repair, reductions in sperm mitochondrial DNA deletions and 8-OHdG were noted in another study, concomitant with elevations in seminal antioxidant levels [129]. Therefore, many clinicians advocate that a 6 months' follow-up duration after varicocele repair is required to achieve sound beneficial effects on OS and sperm DNA fragmentation measures [109].

By contrast, the evaluation of seminal antioxidants after varicocele repair appears to be more complex with contradictory results reported by a number of studies. Nonenzymatic antioxidants such as vitamin C, zinc, selenium, and others have significantly improved postoperatively to normal levels [10, 109, 129, 131]. Also, seminal plasma albumin levels that remain unchanged in the first 3 months post-varicocelectomy, significantly increased in the subsequent 6 months [10]. The exception was with vitamin E. A single study reported normalization of vitamin E levels postoperatively as with other seminal antioxidants [104]. However, a contradictory report revealed a significant reduction in vitamin E levels 3–6 months after varicocelectomy [10]. This discrepancy might be due to the confounding dietary intake factor implicated with vitamin E.

As for the observed responses of enzymatic antioxidants following varicocelectomy, SOD, catalase, and glutathione peroxidase were decreased in one study [109, 116], contrary to another report, documenting a significant increase in the levels of these enzymes at 3 and 6 months following varicocele repair [10]. Hence, further research is needed to uncover this discrepancy.

Interestingly, varicocelectomy was not as effective in alleviating OS in men with normal redox potential preoperatively as compared to those with elevated OS markers [135]. Such results support the rationale that patients with high levels of OS in the testes are better candidates for varicocelectomy.

Effects of Antioxidant Therapy on OS Markers in Varicocele

Oral antioxidant treatment was studied either as an alternative or as an adjuvant treatment to varicocele repair for the management of varicocele-induced infertility. Most of studies used animal models. For instance, using aminoguanidine that is a NOS inhibitor in rats with experimental varicocele significantly reduced the sperm DNA fragmentation levels [31]. In addition, Vitamin E was shown to reduce seminal ROS levels in studies of similar design [136].

Yan et al. [137] used a herbal antioxidant (Jingling) as an adjuvant therapy following varicocelectomy and reported significant reduction in OS in their treatment group. A randomized clinical trial by Cavillini et al. investigated the value of medical treatment on semen parameters and pregnancy rates in 195 varicocele patients and 130 patients with idiopathic oligoasthenoteratozoospermia (OAT) [138]. The authors randomized patients into three groups each containing patients with varicocele or idiopathic OAT. Group 1 received placebo, while groups 2 and 3 received L-carnitine/acetyl-L-carnitine and L-carnitine/acetyl-L-carnitine + cinnoxicam (anti-inflammatory) suppository respectively. After a follow-up period of up to 6 months, the authors reported significant improvements in semen parameters only in groups 2 and 3, with a statistically higher improvement in group 3 as compared to group 2. The pregnancy rates were 1.7%, 21.8%, and 38.0% in groups 1, 2, and 3, respectively. The

authors concluded that antioxidants + anti-inflammatory therapy are beneficial in patients with varicocele and idiopathic OAT [138]. However, further studies are still required to accurately evaluate the long-term effects of medical treatment in varicocele patients. In another study, participants who had underwent varicocelectomy were randomized into four groups, receiving zinc sulfate/folic acid or folic acid or zinc sulfate or placebo [139]. Semen studies revealed reductions in NO concentrations in the treatment groups at 3 and 6 months after varicocelectomy, however, without reaching statistical significance [139]. Additionally, no significant elevations were detected in seminal TAC levels in all groups after surgery. Only SOD activity was significantly improved in the zinc sulfate/folic acid and zinc sulfate groups.

Saalu et al. randomized four groups of rats: Group A served as the control, while Groups B, C, and D of rats were varicocelized [140]. Groups C and D received intraperitoneal and intramuscular treatment of zinc chloride and alpha-tocopherol, respectively. After 56 days, experimental varicocele in Group B rats had a statistically significant decrease in SOD, glutathione peroxidase and catalase activities, lower glutathione content, and higher MDA levels as compared to control animals. Groups C and D showed a significantly increased testicular SOD, glutathione peroxidase and catalase activities, higher glutathione content, and lower MDA levels as compared to those in Group B, and their levels approximated those of the control group [140]. Hence, zinc and vitamin E might have a beneficial effect when used as an adjuvant therapy after varicocele repair. In a study that evaluated the effects of selenium in normal and varicocelized rats, the varicocelized rats showed decreased activity of catalase, glutathione peroxidase, and SOD, and increased levels of MDA in their testicular tissue [141]. The administration of sodium selenite (which supplies the trace element Selenium) normalized these changes in varicocelized rats but had a negligible effect on normal rats, suggesting that selenium may also have a beneficial role in reverting OS measures in a varicocele model.

Antioxidant therapy was studied as monotherapy in varicocele patients as well. Twenty infertile patients with low-grade varicocele were treated with a once daily dose of an antioxidant combination containing vitamins C, E, B12, and B19, L-carnitine, coenzyme Q10, zinc, and selenium for 3 months, revealing significant reductions in their sperm DNA fragmentation levels by about 22.1% [142]. This data may suggest that oral antioxidants can be used to improve sperm DNA integrity especially in patients with low-grade varicocele. Festa et al. [143] evaluated the antioxidant capacity of seminal plasma before and after supplementation of coenzyme Q10 for 12 weeks in patients with male infertility associated with low-grade varicocele. The study demonstrated significant increase in total antioxidant capacity. However, they did not measure the persistence of these changes after discontinuation of therapy. Similarly, another study reported an improvement in the seminal plasma TAC of varicocele patients after 3 months of treatment with coenzyme Q10 [144] further strengthening the usefulness of antioxidant therapy in infertile varicocele patients.

A definitive conclusion on the use of antioxidants in varicocele patients cannot be drawn based on the available data. Furthermore, the few head-head comparative studies between antioxidant therapy and varicocelectomy have either favored that latter [145] or reported analogous benefits for both treatments on semen parameters [146, 147], however, with questionable persistence of the antioxidant effects on the long term. That being said, antioxidant therapy is commonly prescribed by urologists treating men with infertility problems with or without varicocele.

Conclusion

Several mechanisms were proposed to clarify varicocele pathophysiology. OS plays a major role in the pathophysiology of varicocele-related infertility despite not fully understanding its causative mechanisms. Stressful conditions such as heat stress and hypoxia increase OS markers in varicocele patients. The testis responds to OS

through several mechanisms, including the generation of enzymatic and nonenzymatic antioxidants. Failure of these mechanisms can result in spermatogenic dysfunction and infertility. Varicocele repair is a sustainable method to reduce ROS, normalize antioxidant defenses, and restore fertility with results achieved between 3 and 6 months after surgery. Antioxidant therapy use in varicocele patients as monotherapy or adjuvant to varicocelized patients needs further well-designed studies for a definitive indication.

Review Criteria

An extensive search of the literature was done using scientific search engines including Pubmed, Medline, science direct, and Google Scholar. Search criteria included the following keywords: "varicocele," "pathophysiology," "oxidative stress," "reactive oxygen species," and "antioxidants." Data from published papers or book chapters were included.

Multiple Choice Questions and Answers

1. In terms of testicular response to hyperthermia:
   (a) The testicles are naturally kept at a higher temperature than the intra-abdominal measured one.
   (b) **The principal cells in the epididymis, the endothelial cells in the dilated pampiniform plexus, and various testicular cells can produce ROS.**
   (c) NO is only produced by iNOS.
   (d) High levels of NO can interfere with complex III increasing ATP production.
   (e) Heat shock proteins (HSPs) are downregulated in response to hyperthermia.
2. In terms of testicular response to hypoxia:
   (a) Hypoxia decreases the expression of leptin hormone.
   (b) Hypoxia decreases testicular levels of IL-1 and IL-6.
   (c) Hypoxia inhibits glycolysis products such as pyruvate and lactate.
   (d) Hypoxia inhibits the levels of HIF-1α.
   (e) **None of the above.**
3. All of the following are TRUE, except:
   (a) Toxic metabolites from renal or adrenal origin such as urea, prostaglandin (PG) E, PGF2α, and noradrenaline are known to induce cellular OS in several tissues.
   (b) Varicocele patients were reported to have higher levels of cadmium which is a prooxidant that exerts a negative effect on spermatogenesis.
   (c) The redox balance within the epididymis is maintained by the counteracting enzymatic and nonenzymatic antioxidants.
   (d) **An experimental animal model, in which varicocele was induced, resulted in increased proliferation of principal epididymal cells and inhibited carnitine levels, and α-glucosidase activity.**
   (e) Hypoxia, in addition to other cytokines and hormones, can stimulate adrenomedullin secretion.
4. With regard to OS markers in varicocele:
   (a) No conclusive results exist to indicate a significant association between OS measures and varicocele.
   (b) Malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) are examples of direct OS measures.
   (c) **Elevated ROS level is a common finding in varicocele patients with abnormal sperm parameters.**
   (d) The ROS-TAC measure is inferior to ROS or TAC in the evaluation of fertility potential.
   (e) None of the above.
5. With regard to varicocele treatment effects on OS measures:
   (a) Varicocele repair was not found to be significantly associated with improvements in OS measures of sperm DNA fragmentation.
   (b) Antioxidants are superior to varicocelectomy in alleviating OS and hence should be used as first-line treatments.

(c) **Varicocele repair in infertile men has been associated with significant improvements in various biomarkers of male infertility, including OS measures.**
(d) The time required for the beneficial effects of varicocele repair on OS markers is unknown.
(e) Antioxidants supplementation was found to be useless in varicocele patients.

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6 Proteomic and Metabolomic Profile of Semen and Seminal Plasma in Varicocele

Manesh Kumar Panner Selvam
and Ashok Agarwal

Key Points

- Post-translational modification of the sperm proteins and metabolites present in the semen provides valuable information about biomolecules associated with the fertilization potential of the spermatozoa.
- Altered expression of sperm and seminal plasma proteins affects the fertility status of varicocele patients.
- Similarly, changes in the metabolite concentration of sperm and seminal plasma may be a predisposing cause of infertility in varicocele patients.
- Integration of proteomic and metabolomic data using advanced computational and bioinformatic tools allows the identification of novel diagnostic and therapeutic biomarkers.

Introduction

Globally, about one-third of the male infertility cases are diagnosed with varicocele [1] and 15% of men with varicocele are infertile [2]. Varicocele is characterized by abnormal dilation of the pampiniform plexus with the presence of malfunctional valves. In patients with varicocele, testicular function and its environment are disturbed due to the retrograde flow of blood resulting in the state of testicular hyperthermia, hypoxia, and oxidative stress which are detrimental for the production of spermatozoa [3–5]. As a result, semen parameters are altered [6] and varicocele patients exhibit a compromised fertility status.

Male infertility diagnosis in varicocele patients is based on basic semen analysis as per WHO 2010 guidelines [7]. In addition, other advanced sperm function tests are performed to assess the levels of reactive oxygen species (ROS), total antioxidant capacity, and sperm DNA damage/fragmentation [8]. However, these tests do not provide a complete information including the subcellular changes associated with the poor fertilizing ability of the spermatozoa. Advancement in the current "omics" techniques, such as proteomics and metabolomics, has revolutionized the molecular field of sperm biology. Researchers and scientists are able to characterize the structural and functional sperm proteins. This has facilitated the identification of cellular and molecular pathways that are being dysregulated in the

M. K. Panner Selvam · A. Agarwal (✉)
American Center for Reproductive Medicine, Department of Urology, Cleveland Clinic, Cleveland, OH, USA
e-mail: agarwaa@ccf.org

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spermatozoa of infertile patients [9]. Altered expression of sperm proteins and metabolites in infertile patients indicates compromised spermatogenesis or defects in vital sperm functions such as capacitation, hyperactivation, and acrosome reaction, which are essential to the fertilization process.

Proteomic and metabolomic high-throughput platforms are used to identify and select noninvasive biomarkers for the diagnosis of male infertility. Post-translational modification of the sperm proteins and metabolites of the semen provides valuable information on biomolecules associated with the fertilization potential of the spermatozoa. In this chapter, we discuss the proteins and metabolites involved in the regulation of sperm functions that are present in both the cellular (sperm) and fluidic component (seminal plasma) of semen. In addition, we have highlighted the future of proteomics and metabolomics as potential clinical tools for the diagnosis and management of varicocele patients.

High-Throughput Proteomics Techniques

Advanced proteomic techniques are used to identify the complete proteome of a cell. Integration of proteomic data with computational bioinformatic analysis helps in understanding the function of peptides and proteins in cellular pathways. In the current era of proteomics, sperm proteins that are associated with infertility are widely studied [9]. Sophisticated and complex instruments such as liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry were able to overcome the drawbacks of using the conventional 2D gel electrophoresis. The high sensitivity and specificity of these techniques enable to detect maximum number of proteins in sperm [10].

Prior to subjecting the protein samples to proteomic analysis, sperm and seminal plasma proteins are separated and processed for protein extraction. The extracted proteins are resolved either on 1D gel electrophoresis or 2D gel electrophoresis. Later, the gels are cut into pieces and the proteins are digested using trypsin. The sample is then injected into the high-throughput instrument and spectral counts are used to identify and relatively quantify the proteins. Expression of the proteins are measured by comparing NSAF (normalized spectral abundance factor) of each protein [11]. A typical workflow involving the processing of semen samples for proteomics is shown in Fig. 6.1.

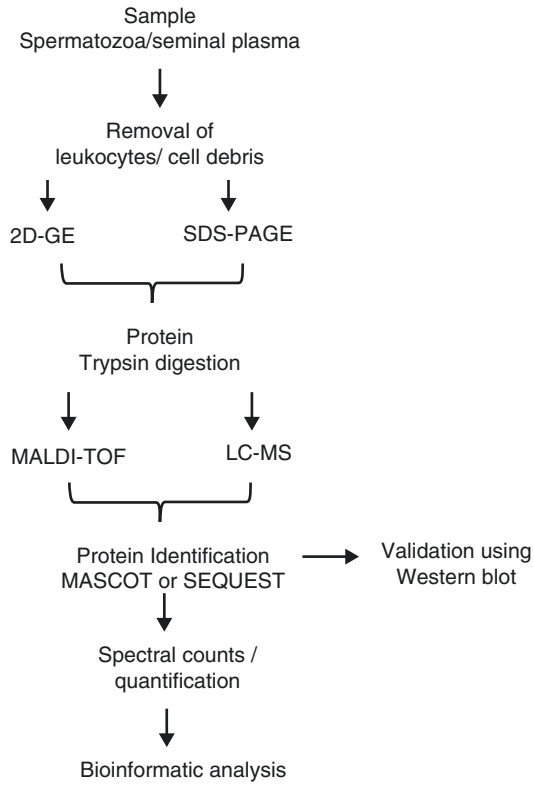

Fig. 6.1 Typical workflow involving the processing of semen samples for proteomics. (Reprinted from Panner Selvam et al. [18]. With permission from Elsevier)

Bioinformatic analysis provides meaningful results from the proteomic data [12]. Gene ontology (GO) analysis of the identified proteins provides information about their localization, distribution, and biological functions. Sophisticated programs such as Ingenuity Pathway Analysis (IPA) and Metacore™ can demonstrate the interaction between proteins and pathways dysregulated due to differential expression of proteins.

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) analysis is commonly performed to display the linking between the proteins [13].

Proteomics and Male Infertility

Semen samples are highly suitable for proteomic analysis and biomarker validation studies. Post-transcriptionally silent spermatozoa depend on proteins to carry out their normal physiological functions. Sperm proteins are associated with molecular pathways such as protein and energy metabolism, post-translational modifications, DNA damage, and oxidative stress response [14, 15]. Apart from sperm proteins, seminal plasma proteins are also essential for the maintenance of functionality of spermatozoa [16]. Seminal plasma secretions are derived from testes and accessory sex glands (Fig. 6.2). Seminal plasma is rich in proteins (35–55 g/L) and semenogelins are present in high abundance (80%). Only 10% of the seminal plasma proteins are contributed by seminal vesicles [17, 18]. Altered expression of the seminal plasma proteins has a direct effect on spermatozoa and may affect sperm homeostasis.

Expression of semen proteins varies from one condition to another. Proteomics was able to demonstrate the differential expression of proteins in semen and its potential use as non-invasive biomarkers in infertile men with abnormal semen parameters. In azoospermic men, the proteins ACPP (acid phosphatase prostate), KLK3 (prostate-specific antigen), CLU (clusterin), AZGP1 (zinc-alpha-2-glycoprotein) and PAEP (glycodelin) were absent in seminal plasma [19, 20]. Drabovich et al. (2013) validated TEX101 (testis-expressed protein 101) as a biomarker in azoospermia and ECM1 (extracellular matrix protein 1) to distinguish nonobstructive azoospermia from vasectomy [21]. In the case of asthenozoospermia, PTPN14 (protein tyrosine phosphatase, non-receptor type 14) was dysregulated [22], whereas CST3 (cystatin-C) was downregulated, and KLK3 and SEMG1 (semenogelin-1) were upregulated in oligoasthenozoospermia (OA) patients [23, 24]. Other proteins associated with

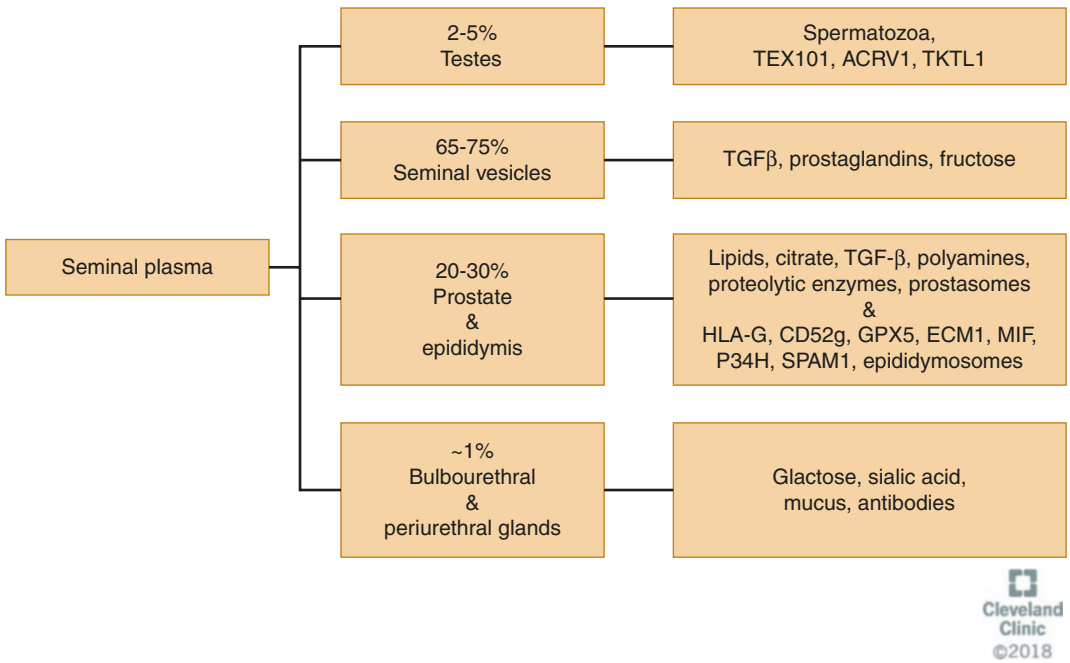

Fig. 6.2 Seminal plasma: contributions of the testes and accessory sex glands and its composition/constituents. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2018. All Rights Reserved)

sperm function such as NPC2 (NPC intracellular cholesterol transporter 2), LGALS3BP (galectin-3-binding protein), LCN1 (lipocalin-1) and PIP (prolactin-inducible protein) were downregulated in oligoasthenoteratozoospermia (OAT) [25].

Proteomic studies conducted by Sharma et al. demonstrated the involvement of DEPs in stress response and regulatory pathways in men with high seminal ROS [23]. The MME (membrane metallo-endopeptidase) protein detected in the seminal plasma of ROS positive men was absent in ROS negative men. Whereas proteins FN1 (fibronectin 1) and MIF (macrophage migration inhibitory factor) were only present in the ROS negative group [23]. Intasqui et al. also reported sperm nuclear DNA damage markers using bioinformatic analysis of proteomic data. SLC2A14 (solute carrier family 2, facilitated glucose transporter member 14), PGK2 (phosphoglycerate kinase 2), ODF1 (outer dense fiber protein 1), CLU, VDAC2 (voltage-dependent anion-selective channel protein 2), VDAC3 (voltage-dependent anion-selective channel protein 3), ZPBP2 (zona pellucida-binding protein 2) and PGC (progastricsin) are reported as potential biomarkers of sperm DNA damage [26, 27].

Varicocele and Sperm Proteomics

In the existing literature, there are very few reports available on sperm proteomics in varicocele patients. Protein profiling was done in normozoospermic men without varicocele and oligozoospermic men with varicocele using 2D gel electrophoresis [28]. Due to the lesser sensitivity of this technique, only 15 DEPs were identified. Molecular pathways involving mitochondrial proteins, cytoskeleton proteins, and heat shock protein were found to be affected in varicocele patients [28]. Another proteomic study by same authors demonstrated the change in the expression of sperm proteins in varicocele patients: pre- and post-varicocelectomy. Expressions of mitochondrial function protein (ATP5D), antioxidant protein (SOD1), and heat shock protein (HSPA5) were significantly increased after varicocele repair [29]. The use of conventional 2D gel electrophoresis was a major limitation in these studies. However, by employing a global proteomic approach (with LC-MS/MS platform) and in-depth bioinformatic analysis, several molecular mechanisms and subcellular pathways affected in varicocele patients were able to be explained (reviewed in [30]).

Varicocele-associated male infertility is a consequence of high state of oxidative stress and mitochondrial dysfunction [31]. Expression of mitochondrial proteins were altered in varicocele patients and linked to the pathophysiology of the spermatozoa with mitochondrial dysfunction [30, 32]. Proteomic profile of the sperm proteins in varicocele patients revealed that 87% of DEPs involved in sperm function and energy metabolism were downregulated in both unilateral and bilateral varicocele patients [33]. Using high-throughput proteome analysis (LC-MS/MS), Samanta et al. (2018) reported 141 mitochondrial proteins in spermatozoa in which 22 DEPs were related to mitochondrial structure and function in varicocele patients. Underexpressions of the ATPase1A4, HSPA2, SPA17, and APOA1 proteins were associated with impaired mitochondrial function [32]. Mitochondrial electron transport chain proteins are regulated by the nuclear transcription factors, and there exists a cross talk between the same (Fig. 6.3). Also, mitochondrial proteins (NDUFS1, ACO2, OGDH, UQCRC2, and IDH3B) interact functionally with each other and are co-expressed in varicocele patients. Underexpression of complex-III of electron transport chain (ETC) (cytochrome bc I complex subunit) in varicocele condition indicates hypoxia-induced oxidative stress [33]. Other ETC (NDFSU1, NADH:ubiquinone oxidoreductase core subunit S1; UQCRC2, ubiquinol-cytochrome C reductase core protein 2; and COX5B, cytochrome C oxidase subunit 5B) and testis-specific protein PDH were suggested as noninvasive biomarker of mitochondrial dysfunction in varicocele patients [32].

Varicocele is encountered on the left side in 90% of unilateral varicocele cases [34]. Comparative proteomic study reported a total of 369 sperm proteins were differentially expressed in fertile men and unilateral varicocele patients.

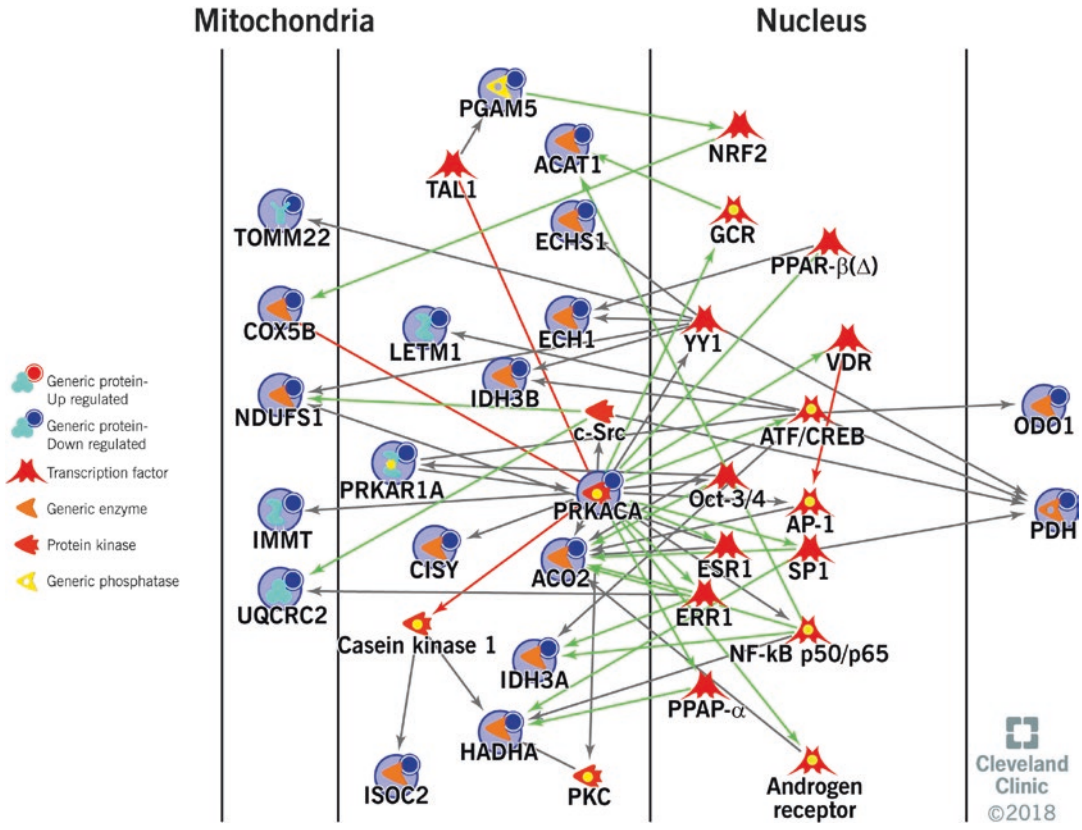

Fig. 6.3 Interaction between the differentially expressed proteins (DEPs) and transcriptional factors in varicocele patients with mitochondrial dysfunction. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2018. All Rights Reserved)

The majority of these DEPs were involved in important cellular molecular functions: ion binding (44.85%), oxidoreductase activity (13.65%), and biological process: small molecule metabolic process (43.73%), response to stress (32.87%), signal transduction (29.25%), and cellular protein modification process (20.33%) [35]. Moreover, altered expression of the sperm proteins had an impact on the molecular pathways, posttranslational modification, free radical scavenging, protein ubiquitination, and mitochondrial dysfunction, which could then affect the normal physiological function of the spermatozoa. A profile of 29 proteins associated with reproductive function (sperm maturation, motility, hyperactivation, capacitation, and acrosome reaction) which are essential for fertilization process were found to be altered in the spermatozoa of unilateral varicocele patients. Based on the coverage of peptide, nine proteins (CABYR, calcium-binding tyrosine phosphorylation regulated; AKAP, A-kinase anchoring protein 5; APOPA1, apolipoprotein A-I; SEMG1, semenogelin-1; ACR, acrosin; SPA17, sperm surface protein Sp17; RSPH1, radial spoke head 1 homolog; RSPH9, radial spoke head protein 9 homolog; and DNAH17, dynein heavy chain 17) associated with fertilization potential of spermatozoa were identified as potential biomarkers for unilateral varicocele patients [36].

Agarwal et al. (2016) demonstrated the differences in sperm proteome profile of bilateral varicocele patients and fertile men. The sperm proteome profile was able to decipher the role of proteins at subcellular level responsible for infertility associated with bilateral varicocele. All together 73 proteins were differentially expressed. The absence of APOA1, underexpression of

mitochondrial import receptor subunit TOM22 homolog (TOM22), and overexpression of protein-glutamine gamma-glutamyl transferase 4 (TGM4) were associated with the molecular pathological changes particularly related to oxidative stress and sperm DNA fragmentation [37]. Also, proteins linked with the reproductive function (such as ODF2, outer dense fiber protein 2; TEKT3, tektin-3; TCP11, T-complex protein 11 homolog; CLGN, calmegin) were aberrantly expressed in the bilateral varicocele patient, thus affecting the fertilization potential of the sperm. Differential expression of sperm proteins ENKUR, enkurin; SEMG1, SEMG2, semenogelin-1; SPAM1, sperm adhesion molecule 1; and CABYR is an indicator of poor semen quality in bilateral varicocele patients [35].

Semen quality is more compromised in bilateral varicocele patients compared to that of unilateral varicocele patients. Comparative protein profiling was able to address the pathophysiology associated with the further damage caused due to bilateral varicocele [35]. 253 DEPs identified between the unilateral and bilateral varicocele are involved in metabolism, apoptosis, and signal transduction pathways. Dysregulation of sperm functions (capacitation, hyperactivation, and acrosome reaction) and reproductive functions (zona pellucida binding and fertilization) in bilateral varicocele patients were more pronounced due to differential expression of GSTM3, glutathione S-transferase Mu 3; SPANX1, sperm protein associated with nucleus; X chromosome; CYB5R2, cytochrome B5 reductase 2; CALGN, calmegin; and PARK7 known as DJ-1 proteins [35]. The majority of these DEPs (>50% of proteins) were involved in the acetylation process and suggest the downregulation of proteasome complex proteins as the predisposing factor for increased DNA damage in bilateral varicocele patients [38]. Acetylation-associated proteins involved in fertilization and acrosome reaction (TALDO1, transaldolase 1; HIST1H2B, histone cluster 1 H2B family member B; GNPDA1, glucosamine-6-phosphate isomerase 1), apoptosis and DNA damage (HSP90AB1, heat shock protein 90 alpha family class B member 1; PPP5C, protein phosphatase 5 catalytic subunit; RUVBL, RuvB-like proteins), and mitochondrial dysfunction and oxidative stress (SDHA, succinate dehydrogenase complex flavoprotein subunit A; PRDX1, peroxiredoxin 1; and GSHR, glutathione reductase) were proposed as posttranslational protein biomarkers in varicocele patients [38].

For an overview of potential sperm biomarkers in varicocele patients based on fertilization, motility and morphology, DNA damage, oxidative stress, mitochondrial dysfunction, see Table 6.1.

Varicocele and Seminal Plasma Proteomics

In addition to sperm proteins, the seminal plasma proteome also plays a key role in determining the fertilization capacity of the sperm [39]. Seminal plasma provides a favorable environment for the maturation of spermatozoa, and its proteome reflects the functionality of the male reproductive tract. It carries important information regarding testicular function, as about 10% of the proteins found in seminal plasma originate from the testes [40] (Fig. 6.2). Seminal plasma proteins support various fertilization processes such as hyperactivation, capacitation, acrosome reaction, and sperm-oocyte interaction [41, 42]. It contains approximately 30% of the sperm proteins, which reflect the functional state of the spermatozoa [17]. Seminal plasma serves as a potential source for protein biomarkers, especially the differentially expressed proteins (DEPs) involved in the pathophysiology of male infertility can be used as predictive biomarkers in its diagnosis [43]. Potential seminal plasma protein biomarkers are listed in Table 6.1.

Protein Signature of Varicocele in Adults

A first report on seminal plasma proteomics in adult varicocele patients using 2D gel electrophoresis (2D SDS–PAGE) technique was published

Table 6.1 Potential protein biomarkers in spermatozoa and seminal plasma of varicocele patients

Function	Sample	Potential biomarkers	Study
Fertilization	Sperm	TALDO1, HIST1H2B, GNPDA1	Selvam et al. [38]
		TCP11	Agarwal et al. [37]
		HSPA2	Agarwal et al. [33]
		CRISP2, CALGN, SPAM1	Agarwal et al. [35]
		APOPA1, ACR, SPA17, TGM4, HIST1H2BA	Agarwal et al. [36]
	Seminal plasma	PSA	Zylbersztejn et al. [47]
Motility	Sperm	AK7	Agarwal et al. [33]
		TEKT3	Agarwal et al. [37]
		CABYR, AKAP3, SEMG1, DNAH17, ODF2	Agarwal et al. [36]
Morphology	Sperm	RSPH1, RSPH9	Agarwal et al. [36]
		SPANXB1	Agarwal et al. [35]
		CCT6B	Agarwal et al. [33]
DNA damage	Sperm	HSP90AB1, PPP5C, RUVLB	Selvam et al. [38]
	Seminal plasma	DNASE1	Belardin et al. [48]
		BCL2, BAX	Mostafa et al. [50]
		SMG1, IGFBP-3	Zylbersztejn et al. [47]
Oxidative stress	Sperm	PARK7	Agarwal et al. [35]
		SOD1	Hosseinifar et al. [29]
	Seminal plasma	NELFE	Del Giudice et al. [48]
Mitochondrial dysfunction	Sperm	NDFSU1, UQCRC2, COX5B, PDH	Samanta et al. [16]
		SDHA, PRDX1, GSHR	Selvam et al. [38]
		PKAR1A, AK7, CCT6B, HSPA2, ODF2	Agarwal et al. [33]
		DLD	Agarwal et al. [36]
		ATP5D	Hosseinifar et al. [29]

in 2012 [44]. Their group reported of 95 proteins that were differentially expressed in the seminal plasma of cigarette smoking adult varicocele patients. Moreover, proteins involved in inflammatory response, proteolysis and regulation of apoptosis, sperm maturation, and sperm-oocyte fusion were dysregulated in these patients [44]. Nitric oxide metabolism and tetratricopeptide repeat domain-binding functions were also more enhanced in adult varicocele patients [45]. These alterations in the seminal plasma proteome mark the deleterious effect of varicocele on semen quality and sperm function integrity in adult males.

Protein Signature of Varicocele in Adolescents

Varicocele also occurs with a prevalence of 6–26% in adolescents and 15% in age group 11–19 years old [46]. In adolescents with varicocele having poor semen quality, the seminal plasma proteins associated with normal physiological function of spermatozoa are differentially expressed. Proteins associated with sperm motility and capacitation such as SEMG I and PSA were overexpressed and underexpressed, respectively, in seminal plasma of adolescents with varicocele [47]. Belardin et al. (2016) reported that the seminal plasma proliferative or apoptotic equilibrium is altered in varicocele patients [48]. Insulin-like growth factor-binding protein 7 (IGFBP7) associated with proliferative process was overexpressed, whereas deoxyribonuclease-1 (DNASE1) involved in regulation of apoptosis was underexpressed in the seminal plasma of adolescents with varicocele [48]. Furthermore, proteomic analysis by the same group revealed that seminal plasma was enriched with immune response proteins leading to a chronic inflammatory reaction in adolescents with varicocele [49]. These changes affected testicular functions in adolescents with varicocele leading to decreased semen quality.

Protein Signature After Varicocelectomy

A drastic change in the expression profile of proteins has been observed in the seminal plasma of post-varicocelectomy patients. The proteome profile of seminal plasma in post-varicocelectomy revealed 38 proteins were uniquely expressed. Molecular pathways such as response to oxidative stress, gluconeogenesis, and protein stabilization were enriched in post-varicocelectomy patients. Overexpression of DJ-1, parkinsonism-associated deglycase; S100-A9, S100 calcium-binding protein A9; SOD, superoxide dismutase 1; ANXA1, annexin A1; G3P, glyceraldehyde-3-phosphate dehydrogenase; and MDH, malate dehydrogenase, in seminal plasma can help retain the homeostasis post-varicocelectomy [45]. Decreased expression of negative elongation factor E (NELFE) indicates a decreased state of oxidative stress, whereas increased expression of transglutaminase-4 infers the sperm-binding activity was retained in post-varicocelectomy patients [47].

Other validation studies of specific seminal plasma proteins were performed and analyzed to develop noninvasive biomarkers for the diagnosis of varicocele-associated male infertility. Apoptotic markers B-cell lymphoma 2 protein (BCL2) and BCL2-associated X protein (BAX) expression are decreased and increased, respectively, in the seminal plasma of varicocele patients. BAX was negatively correlated with sperm concentration, motility and normal sperm morphology [50].

Metabolomics and Male Infertility

Metabolomics deals with the nontargeted profiling of a complete set of small molecules (<1 KDa) such as hormones, signaling molecules, and secondary metabolites [51]. The complexity of metabolites is less compared to proteins and mRNA transcripts present in the cell. Comparatively, metabolites provide more information than proteins, mRNA transcript, and genes as they form the final product of the cellular process. Thus their effect on the biological system are direct [52]. Targeted and nontargeted metabolomic approaches are widely used. Several high-throughput platforms such as Raman spectroscopy and ^{1}H nuclear magnetic resonance (NMR) spectroscopy, near-infrared (NIR) spectroscopy, electrospray ionization mass spectrometry (ESI-MS) [53], and direct-injection mass spectrometry (DI-MS) [54] are available for performing metabolomic profiling on a small quantity of sample. The samples are stored at −80 °C to stop the metabolomic activity.

The metabolome is the final product of the genome. Biofluids are the most suitable samples for metabolomic profiling [55]. Seminal plasma is a good medium for assessing the fertility status of an individual. It reflects the changes and alterations in the spermatozoa at the subcellular/molecular level [56]. Metabolomic profiling of the seminal plasma provides the metabolic features of the semen quality and the pathophysiology condition associated with it. Abnormal metabolic changes in the seminal plasma is associated with the pathophysiology of male infertility [57]. Comprehensive profiling of the metabolites in semen (spermatozoa/seminal plasma) reveals the global change in the small molecular signature of metabolites. For the management of male infertility, it is crucial to identify the change in the metabolomic profile of semen [58]. Using bioinformatic tools, the pathways linked with the pathophysiology of sperm are able to be determined. Whereas, chemometrics analysis is very much useful in differentiating the infertile from fertile men [59].

Nuclear magnetic resonance (NMR) spectroscopy technique for metabolomic profiling of seminal plasma indicated lysine as a potential biomarker to diagnose idiopathic infertility in men [60]. Seminal plasma metabolites such as valine, 2- hydroxyisovalerate, lysine, hippurate, and fructose levels are lower in idiopathic infertile men [60]. Bonechi et al. (2015) used the NMR spectroscopy and principal component analysis (PCA) approach to discriminate semen samples based on infertility conditions. Similarly, patients with leukocytospermia were found to be grouped together [61]. Metabolomic profiling

using Raman spectroscopy can diagnose asthenozoospermic semen samples from normozoospermic samples with 83% of accuracy [62].

In asthenozoospermia patients, metabolites such as 5α-cholesterol and 7-ketocholesterol levels are increased and involved in oxidative stress mechanism [56]. Later, Gilany et al. (2017) used gas chromatography-MS and advance chemometrics analysis to demonstrate that 36 discriminatory metabolites present in seminal plasma to distinguish the testicular sperm extraction (TESE)-positive and TESE-negative nonobstructive azoospermia samples. Only five metabolites, dimethyl-(1S)-bicyclo(3.1.1)hept-2-ene-2-methanol, 2-pyrrolidineacetic acid, and 4,5-dimethoxy-1,2-benzenedicarboxylic acid, are identified in the database of human metabolome (version 3.6). An increase in the –CH functional group in seminal plasma is an indicator of oxidative stress biomarker in men with unexplained infertility [63]. Profiling of seminal plasma lipids using the metabolomic approach in spinal cord injury (SCI) patients revealed the presence of metabolites associated with nucleotide biosynthesis, and response to hydrogen peroxide pathways. Signal transduction pathway is severely affected in the SCI patients [64]. In case of unexplained male infertility, seminal plasma metabolite profiling can identify patients with 82% and 92% of accuracy and specificity, respectively.

Future of Proteomics and Metabolomics

Rapid progress in the proteomic (LC-MS/MS, MALDI-TOF) and metabolomic (Raman, ^{1}H NMR, NIR, ESI-MS) techniques over the last 5 years has geared the "omics" research to validate the proteins and metabolites as potential diagnostic and therapeutic biomarkers for male infertility. Initially, several challenges were faced in sperm and seminal plasma proteomics pertaining to the complexity of the sample, processing of sample for mass spectrometry analysis, quantification of proteins, and identification of post-translational modifications (PTMs) [65]. Simplification of the proteomic techniques by employing protein enrichment strategies and targeted proteomic approach can detect the low abundance proteins and PTMs (glycosylation, phosphorylation, acetylation, and methylation) effective in sperm and seminal plasma. On the other hand, profiling of metabolites and identification of the metabolic signature of seminal plasma in different male infertility conditions can improve the personalized treatment strategy for couples undergoing assisted reproductive technology (ART). Furthermore, validation of metabolites in large sample sizes may lead to the development of novel diagnostic tests for male infertility patients.

Apart from the sperm and seminal plasma protein studies, the focus has currently shifted to understand the physiological function of the exosomes. Yang et al. in 2017 profiled the seminal exosomal proteins in fertile donors. Exosomal proteins were associated with protein metabolism, energy pathway, and transport [66]. Differential screening of exosomal proteins in infertile male patients may serve as a potential biomarker in assessing the functional status of exosomes in seminal plasma. Recently, we have identified the alterations in seminal plasma protein associated with exosome functions in varicocele patients. Exosome-associated proteins ANXA2 and KIF5B may serve as potential protein biomarkers of exosomal dysfunction and exosome-mediated infertility in varicocele patients [67].

Proteomics and metabolomics are a technology-driven field and rely on bioinformatic analysis. Advancement in computational tools and user compatible data analysis tools such as IPA, Metacore, Cytoscape, and Reactome makes the interpretation of results more versatile and feasible. Implementation of these techniques into a clinical set up depends on powerful meta-analysis of biomarker validation results. It is anticipated that the future of male infertility diagnostics and therapeutics depends on the effective integrated analysis of all the "omics" (genomic, proteomic, and metabolomic) data to identify accurate and reliable biomarkers for a specific infertility condition.

Conclusion

Besides the advanced tests performed to determine oxidative stress and DNA fragmentation, molecular biomarkers can be promising in noninvasive diagnosis of pathology associated with male infertility. Proteomic and metabolomics must be considered as two complementary "omics" tools to investigate the biomarkers of male infertility. Although the proteomic and metabolomic results thus far seem promising, validation of the biomarkers in larger sample sizes using Western blot or ELISA will definitely strengthen these "omics" results. In-depth "omics" studies on seminal exosomes can help in developing new diagnostics and therapeutic strategies for treating exosome dysfunction in infertile men with varicocele.

Review Criteria

Extensive literature search was performed on search engines such as PubMed, Medline, Google Scholar, and Science Direct databases. Information from the studies published until June 2018 were extracted. The literature search was limited only for the articles written in English language. "Varicocele" and "proteomics" and "metabolomics" were the two main key terms used for conducting literature search. Other keywords used to retrieve relevant articles were "male infertility," "biomarkers," "seminal plasma and proteomics," "varicocele and biomarkers," and "metabolomics and male infertility and varicocele." Also book chapters and data published in scientific meetings relevant to varicocele and proteomics were included in this review.

Multiple Choice Questions and Answers

1. Semenogelin is present in?
   (a) Saliva
   (b) **Semen**
   (c) Vaginal fluid
   (d) Blood
2. Which of these techniques is used for protein profiling?
   (a) Western blotting
   (b) ELISA
   (c) PCR
   (d) **LC-MS/MS**
3. Which of the following computational tools is used for analyzing metabolomic data?
   (a) **Bioinformatics and chemometrics**
   (b) Virtual modelling of metabolites
   (c) Simulation tools
   (d) Metabolite docking softwares
4. In varicocele patient the protein profiles are significantly altered in?
   (a) Seminal plasma alone
   (b) Sperm alone
   (c) **Sperm and seminal plasma**
   (d) None of the above
5. Suggested protein biomarkers of mitochondrial dysfunction in varicocele patients?
   (a) Semenogelins
   (b) DNASE1
   (c) KIF5B
   (d) **NDFSU1, UQCRC2, COX5B, PDH**

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Experimental Varicocele

7

Fatima Kazue Okada, Renata Cristina de Carvalho, and Ricardo Pimenta Bertolla

Key Points

- Experimental studies allow isolation of the effect of varicocele for the study of male infertility.
- Experimental varicocele may compare the consequences of varicocele by sham-control group analyses.
- Partial ligation of the left renal vein is the main method to induce experimental varicocele, with consequent increased left spermatic vein caliber, thus mimicking the human varicocele.
- Most experimental varicocele studies have been performed in rats.
- Experimental studies may also be used to assess the effect of varicocele in association with comorbidities and/or drugs.

Introduction

Prevalence of Varicocele

Varicocele is characterized by the presence of dilated veins in the pampiniform plexus with venous reflux due to dysfunctional or absent valves. Its prevalence in the adult male population is about 15%. However, 40% of individuals with primary male infertility and 80% of individuals with secondary male infertility present with varicocele, which has led to its association and suggestion as a progressive condition [1–5].

Varicocele is more commonly observed, and more important in terms of its size, on the left side. This occurs due to some anatomical characteristics. While the right internal spermatic vein flows into the inferior *vena cava* at an oblique angle, the left internal spermatic vein flows into the left renal vein, which is smaller in caliber, and at a right angle. This leads to an increased hydrostatic pressure on the left side [6, 7]. Furthermore, the left renal vein is intermittently compressed by the superior mesenteric artery and the abdominal aorta, in a phenomenon denominated as the nutcracker effect [8]. These factors may cause blood to reflux into the pampiniform plexus, causing varicocele [9]. This condition in turn can lead to scrotal hyperthermia, hormonal disturbance, testicular hypoperfusion, hypoxia, and renal and adrenal metabolic reflux of toxic metabolites [10].

Despite varicocele being well described in men, studies have shown a lot of variation. This variation in many cases is attributed to individual variation; however, one cannot rule out cofactors such as comorbidities, eating habits, smoking, and exposure to environmental toxicants, among others.

F. K. Okada · R. C. de Carvalho · R. P. Bertolla (✉)
Department of Surgery, Division of Urology, Sao Paulo Federal University, Sao Paulo, SP, Brazil

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In order to deal with this difficulty, experimental models have been used. Varicocele occurs naturally only in primates; therefore, laboratory animals have been commonly used for the induction of varicocele.

Advantage of Using Animals

Experimental models have been used in different fields of medicine due to the possibility of controlling the environment factors that may interfere with the study results. When animals are used, most external factors can be controlled, such as: the environment humidity, air quality, ventilation, food and its quality, cage substrate and its quality, water and its quality, noise, and light [11, 12]. For the same reason, varicocele has been studied in animals, despite the high incidence of this condition in humans. Beyond that, when the experimental model is used, some further analyses are possible to be carried out, such as tissue evaluation, extraction of germ cells for in vitro culture, pregnancy and fertility rates in multiple females, and/or birth rates [13, 14].

Finally, experimental models are advantageous because of the possibility of a true control (control-sham groups). Because animals in the control-Sham group are submitted to the same conditions and procedures as animals in the study group, discomfort and distress of the procedure are considered and removed as confounding factors.

Care in Handling Animals

Before creating a project with experimental models, it is important to obtain previous knowledge about the species that can be used, and the lineages most recommended for the study. It is important to bear in mind that there are anatomical, physiological, and behavioral differences between species that may interfere in the research development, leading to compromised results [15]. This step is necessary to avoid poorly designed projects and mainly unnecessary loss of animals.

All projects that use the experimental models must be approved by the Institutional Animal Care and Use Committee [16]. Furthermore, laboratory animals must be kept under controlled conditions and constant monitoring, avoiding stress factors which could confound results.

It is of utmost importance for a good research development that the main researcher has experience in animal handling that will be used. Moreover, all researchers, who will participate in the project without experience, should undergo training before the start of the project to avoid causing discomfort, distress, and pain to the animals due to misconduct by inexperience with the animal used in the study [15]. This step is necessary for researcher to acquire confidence and expertise to avoid intercurrences during the procedures.

Also, selecting the ideal animal model to research allows for reduction of variability. Therefore, researchers must consider [17]:

- According to the literature, what species will produce the most relevant and useful results that will most closely resemble human characteristics?
- Which species is most appropriate for the study, considering anatomical, physiological, and behavioral characteristics?
- Which species will use the smaller number of animals?

Animal Models Used

According to the literature, experimental varicocele can be induced in a variety of animals, such as dogs, rabbits, monkeys, and rats. The dog is the animal least used in the study of varicocele; induction is achieved by occlusion of the left renal vein, by destruction of the left testicular vein valves [18], or by occlusion of the pampiniform plexus with silicone [19]. Rabbits have been used in varicocele studies, although their use has decreased significantly. In these animals, varicocele is achieved by partial ligation of the left lumbotesticular trunk [20]. In addition to the animals mentioned above, it is possible to perform varicocele

induction in Rhesus monkeys. Induction in non-human primates is usually performed by partial constriction in the left renal vein between the inferior vena cava and the adrenal vein [21].

Among all experimental models, the most used to study varicocele is the rat. Induction of varicocele in rats is performed in a similar manner as described in monkeys, by partial constriction of the left renal vein [18, 22, 23]. Rats often used for this procedure are *Wistar* and *Sprague-Dawley*. This occurs because their anatomical and physiological traits are well characterized, they are docile and easy to handle, they present excellent reproductive performance, short gestation periods, a short life cycle, and are fairly easy to acquire [24]. In this chapter, we will mainly discuss varicocele induction in rats.

Description of Varicocele Induction

Varicocele induction in rats is performed by reducing the left renal vein caliber, which eventually leads to local blood reflux. The compression site must be in the left renal vein between the caudal vena cava and the left spermatic vein.

The varicocele induction surgery is initiated with anesthetic and analgesic administration to the animal. After verifying anesthetic effects by the loss of reflex movement, the surgical site should be prepared by abdominal trichotomy and antisepsis. The rat must then be immobilized in supine position. Surgical procedure is then initiated by a 3 cm longitudinal incision along the *linea alba*. With access to the abdominal cavity, the small intestine and other abdominal contents must be pushed to the side or pulled outside the cavity in order to allow visualization of the left renal vein.

At this point, it is already possible to observe the left kidney, the left renal vein, the caudal vena cava, and the left spermatic vein. The next step is the most important and delicate of the surgery procedure. Using a curved watchmaker forceps, the left renal vein is dissected in two sites: one located cranially and the other caudally to the left renal vein. Each dissection site should have a depth of 3–4 mm. After following all these steps, the forceps must be inserted into one of the dissection sites to create an opening in the dorsal portion of left renal vein toward the other dissection site. It is extremely important to be careful not to damage the left renal vein or other blood vessels during this delicate procedure. These sites will allow for wrapping of a polyester/cotton blue nonabsorbable suture for partial ligation.

To standardize compression, a flexible segment of epidural catheter (diameter 0.85 mm) is used. It is placed parallel to the renal vein, which is then ligated. The suture is wound around the vein and the catheter, and two knots must be tied, with care so as not to completely occlude the left renal vein. The catheter is then removed and the excess of nonabsorbable cotton/polyester suture snipped. Abdominal content is then moved back into place, and abdominal muscle and skin layers are sutured with a 4.0 nylon fiber (Table 7.1).

Material Used for Induction

The surgery should be planned in advance to avoid intercurrences. The surgical environment, in which the procedure will be carried out, must be organized as every surgery room with the following established standards: clean room as well as good lighting and ventilation [25]. On the day of varicocele induction, all surgical material should be disposed in a way that facilitates the steps of the procedure (Fig. 7.1).

Table 7.1 Summary of the surgery steps

1. Administer anesthetic and analgesic
2. Perform abdominal incision
3. Displace the small intestine to the outside or push abdominal content to the side
4. Observe the veins: left renal, left spermatic and caudal vena cava
5. Dissect around the left renal vein, between spermatic vein and caudal cava
6. Dissect dorsally the left renal vein to separate it from left renal artery
7. Decrease the left renal vein caliber with the twisted polyester/cotton blue nonabsorbable suture, using a catheter as a guide
8. Relocate the small intestine in the abdomen cavitary
9. Suture abdominal muscle and skin layers

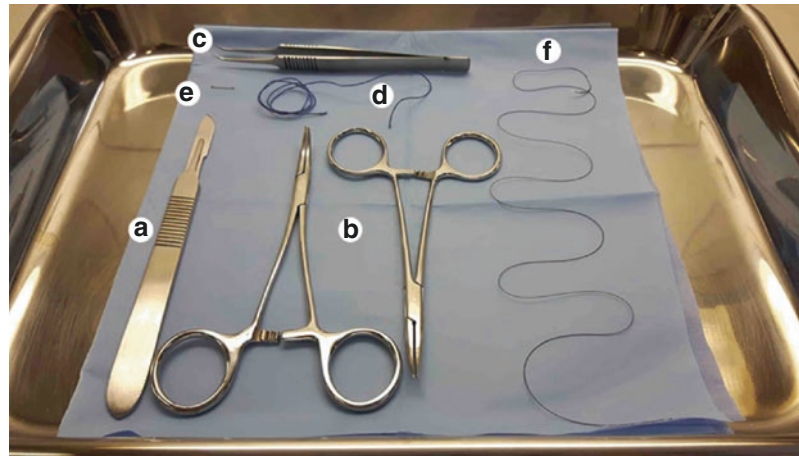

Fig. 7.1 Surgical instruments used for varicocele-induced. (**a**) Scalpel no. 10, (**b**) curved hemostatic forceps, (**c**) curved watchmaker forceps, (**d**) twisted polyester/cotton blue nonabsorbable suture, (**e**) flexible segment of epidural catheter (diameter 0.85 mm), (**f**) nylon 4.0 suture with needle cti 3/8 circle triangle 2.0 cm

Below are some instruments indicated/required to varicocele induction:

- Anesthetic and analgesic

Surgery is performed under anesthesia, and analgesia should be performed, as per Animal Care and Use Committee (ACUC) guidelines. Because the surgery lasts approximately 30–40 minutes, it is considered a medium-sized surgery. Commonly used anesthetics are pentobarbital associated with xylazine or ketamine associated with xylazine, both administered intraperitoneally, or isoflurane, by inhalation.

- Surgical instrumentation

A *scalpel no. 10* is used to perform a craniocaudal longitudinal incision after asepsis for opening the abdominal cavity (Fig. 7.1a). *Curved hemostatic forceps* are used to clamp the animal skin and muscle. It is important to maintain the abdominal cavity open (Fig. 7.1b). Curved watchmaker forceps 45′-angled no. 5 is used to dissect around the left renal vein (Fig. 7.1c).

The left renal vein caliber is reduced with a twisted polyester/cotton nonabsorbable suture and a flexible segment of epidural catheter (diameter 0,85 mm) (Fig. 7.1d, e). The catheter is used to standardize the compression and to avoid strangulation of the left renal vein. The twisted polyester/cotton blue nonabsorbable suture is used to suture the left renal vein. The nylon 4.0 suture with needle cti 3/8 circle triangle 2.0 cm is used to suture the abdominal muscle and skin (Fig. 7.1f).

Analysis After Surgery

After the surgical procedure and all post-surgery care, the outcome of induction can be verified. In physiological conditions, the left spermatic vein caliber is 0.15–0.2 mm (Fig. 7.2a); however, after 30 days of the varicocele induction, its caliber increases gradually, up to 1.5 mm (Fig. 7.2b) [26].

Some techniques can be used to verify the left renal vein, such as venography and ultrasonography. Venography is a procedure which requires intravenous administration of a contrast followed by an animal's pelvic radiography. Another way to verify induction is ultrasonography. With both techniques, it is possible to verify if there is an increase in the left renal vein caliber. However, analysis of the left renal vein can also be performed by direct visualization during euthanasia (Fig. 7.2b).

The time required to analyze the effects of induced varicocele will depend on the cell or tissue that will be analyzed. To study the germ cells, it is necessary to wait at least 53 days (time of complete spermatogenesis cycle) after onset. To study spermatozoa, it is recommended to wait an additional 12 days, to account for epididymal maturation. It is important to bear in mind that

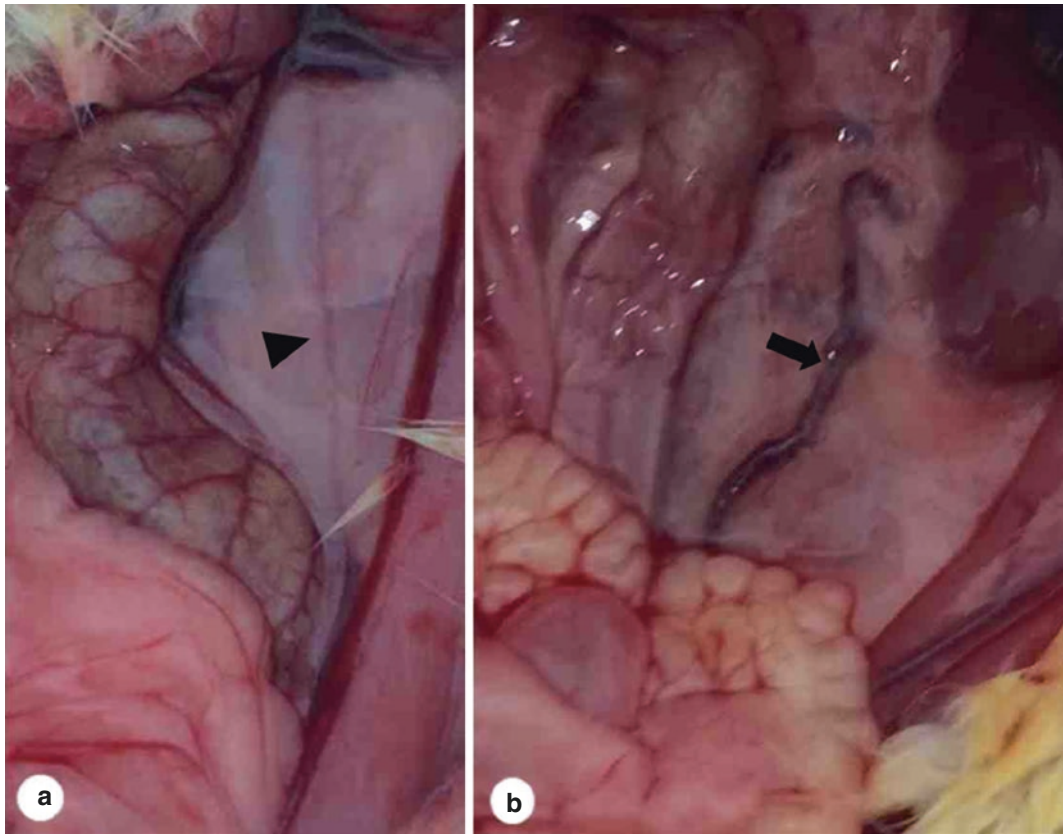

Fig. 7.2 Rat abdominal cavities. Note left spermatic vein before induction of varicocele (**a**) and 60 days after surgery procedure (**b**). Head arrow: left spermatic vein without intervention. Arrow: left spermatic vein with increased caliber after varicocele induction

varicocele induction can take XX to YY days, and this period should be added to the waiting period.

Results Obtained with Animals Submitted to Varicocele Induction

After confirmation of varicocele induction, many analyses can be performed in these animals, such as tissue analysis, cell evaluation, molecular and hormonal study, and other analyses.

The varicocele-induced animal has been used to study varicocele for quite some time. Tissue analysis has been done to evaluate seminiferous tubules and germ cells. It is considered a gold standard in the study of the varicocele effect, because it allows direct observation of the spermatogenic cycle. Results have shown seminiferous epithelium degeneration in varicocele-induced animals [27, 28]. Besides, the epididymis has also been studied in this model, presenting with decreased caliber, thus demonstrating the influence of the varicocele in this organ [29].

The varicocele-induced model has also enabled cellular studies. Sperm are the most studied cells in varicocele-induced animal; beyond them other cells can be studied, such as germ cells and interstitial cells. Studies have shown a decrease in sperm concentration, motility, morphology, and viability after varicocele induction [30]. Moreover, sperm present with protamine deficiency and increased DNA fragmentation [31]. Some studies have suggested this may arise from oxidative stress. Blood plasma from the spermatic vein presents with increased reactive

oxygen species levels [27]; with no differences observed in antioxidants levels [32]. Because HSPA2, e-cadherin, and α-catenin levels have been shown to be altered, pathways involved in heat shock have arisen as of special note in the experimental varicocele [27, 30]. Induced varicocele has also been shown to produce decreased in vitro fertilization and lower embryo cleavage rates [32].

In terms of assessing effectiveness of treatment of varicoceles, a few studies have proposed treating these animals with different types of antioxidants, showing improvement in testicular histology, decrease in sperm DNA fragmentation, increase in sperm motility, and improvement in mitochondrial activity, in acrosome integrity, and in testicular oxidative stress [33–35]. An experimental varicocelectomy has also been tested, and authors also demonstrated improvement in sperm DNA fragmentation rates after reversion of the varicocele [29].

Finally, some studies have sought to elucidate how varicocele would act as a co-morbidity. Varicocele potentiated the effects of nicotine on testicular damage [36], while another study demonstrated a decrease in testicular weight and in epididymal sperm concentration when nicotine was administrated in varicocele-induced animals [37].

Conclusion

Varicocele is a disease that is highly prevalent in men, and is the main treatable cause of male infertility. However, varicocele studies in humans are limited in terms of how much they can answer, because of the high individual variability in fertile potential of men, and because many interventions are difficult to achieve in humans. With the purpose of eliminating these difficulties, animal models are used in a surgical varicocele induction model that results in pampiniform plexus dilatation. Most experimental studies have been performed in rats, which presents physiological characteristics similar to humans, mimicking the effect of this condition on male fertility. Most studies have demonstrated that varicocele leads to altered semen quality, sperm functional integrity, and semen oxidative stress, and that treatment is effective in reverting, or dealing with, some of these effects.

Review Criteria

This chapter was elaborated using the PUBMED database. Manuscripts were selected by keywords: "animal," "varicocele," "rat," "mouse," "dog," "monkey," "rabbit," "experimental varicocele," "sperm," "testis," "epididymis," "left spermatic vein," "animal research," and "animal ethic."

Relevant references cited in the selected manuscripts were considered in the chapter.

All considered manuscripts were written in English language.

The manuscript publication period was not taken into consideration.

Multiple Choice Questions and Answers

1. How can the best laboratory animal be chosen for the induction of varicocele?
   (a) Select the experimental model already used in your laboratory for other approaches.
   (b) Choose the available species.
   (c) **Select the experimental model with anatomical and physiological characteristics favorable to the study.**
   (d) Use a species with unknown anatomical and physiological characteristics.
   (e) Select the cheapest model.
2. Research performed in experimental model should be conducted in a correct way. Why is researcher experience important?
   (a) To cause distress to the animal.
   (b) **To conduct the research without intercurrence.**
   (c) To provoke distress and discomfort to the researcher.
   (d) To generate pain to the animals.
   (e) To hinder the research development.

3. What is the best way to induce varicocele in laboratory animals?
   (a) By decreasing left spermatic vein caliber.
   (b) **By decreasing left renal vein caliber.**
   (c) By increasing left renal vein caliber.
   (d) By decreasing right renal vein caliber.
   (e) By increasing right spermatic vein caliber.
4. What analysis may be easily made in animals that are difficult to perform in humans?
   (a) To analyze the sperm.
   (b) To analyze the seminal plasma.
   (c) **To analyze the seminiferous tubule.**
   (d) To evaluate the spermatic vein caliber
   (e) To evaluate the varicocele grade
5. Animals have been used as experimental models for the study of varicocele. What is their main advantage?
   (a) **To remove the frequent comorbidities in humans.**
   (b) Impossibility of comparison with other groups.
   (c) To promote experience with animal handling.
   (d) To know the anatomical and physiological characteristics of the animal.
   (e) Possibility of using animals in an unlimited manner.

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Part II

Clinical Evaluation of Varicocele

8 Epidemiology of Varicocele in Pediatric, Adolescent, and Adult Populations

Mohannad Alharbi and Armand Zini

Key Points

- Varicocele is encountered in 15% of healthy men and up to 35% of men with primary infertility.
- Varicocele prevalence in the pediatric and adolescent population varies greatly according to the age group and the grade of varicocele at presentation.
- The rise in prevalence in post-pubertal boys has been linked to the venous incompetence that occurs during testicular development.
- Varicocele is a "progressive" disease resulting in a time-dependent decline in fertility potential.
- There appears to be an association between aging and varicocele in some reports.
- There is a relationship between varicose veins of lower extremities and varicoceles, and an inverse relationship between BMI and varicocele exists.

Introduction

Varicocele is defined as tortuous and/or dilated veins of the pampiniform plexus in the scrotum. It is commonly found on the left side; however bilateral varicoceles are not uncommon [1]. An isolated right-sided varicocele is very rare and should prompt investigation for an underlying retroperitoneal tumor. Varicoceles are encountered in 15% of the normal adult male population. Its prevalence in men with primary infertility is 35%, and up to 80% of adult male with secondary infertility suffer from this condition [2].

Investigators have suggested three theories that explain the dilatation and reflux in varicocele. First is the differences in angles of insertion and length between the left and right internal spermatic veins. Second is the absence or incompetence of venous valves. Third is the "nutcracker" phenomenon, where there is a compression of the left renal vein between the aorta and superior mesenteric artery which can cause outflow stasis and increased hydrostatic pressure [3–5].

The association between varicocele and impaired spermatogenesis has been well established. The most widely known theory is scrotal hyperthermia, through which varicocele disrupts the countercurrent heat exchange system and affects spermatogenesis and endocrine function [6–9]. It has been shown that heat shock proteins have a protective effect during heat stress [10].

M. Alharbi (✉)
Division of Urology, Department of Surgery, McGill University, Montreal, QC, Canada

Department of Urology, College of Medicine, Qassim University, Qassim, Saudi Arabia

A. Zini
Division of Urology, Department of Surgery, McGill University, Montreal, QC, Canada

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The reflux of adrenal and renal metabolite such as urea, prostaglandins E & F, and norepinephrine can disrupt spermatogenesis [4, 11].

The association between male fertility potential and varicocele has not been fully established. Up to the present time, several studies have shown a relationship between varicocele and infertility. However, most studies have examined highly selected populations (e.g., infertile men) representing an important reason for the debate concerning the association between varicocele and male infertility [12].

Physical examination is critical for the diagnosis of varicocele. Patients should be examined in a warm room. Clinical varicoceles are graded based on Dubin grading system [13]. Despite the development of a grading system for varicocele, it is important to note that there is variability in the results of epidemiological studies due to many reasons: [1] differences in the populations included in the studies, variability in the methods used to detect varicocele across different studies, and the geographical source of the published studies.

It is imperative to understand the epidemiological aspect of varicocele which helps in counseling of the patients, identifying risk factors and potential associations, and appreciating the progressive nature of varicocele and its effect on fertility.

The varicocele prevalence differs according to the age group and the setting of the conducted study (epidemiological vs. clinic-based studies), which can be a source of bias that one needs to be aware of when interpreting the results of such studies.

Prevalence of Varicocele in the General Male Population

The early epidemiological studies reported the prevalence of varicocele at 4–25% with an average of 15% [1, 14–17]. These studies were based on school boys and military recruits. Clarke reported the prevalence of varicocele to be 8% after including 275 men from a normal population [15]. Steeno et al. reported varicocele prevalence in 4067 boys and college students aged 12–25 years to be 14.7% [16]. Similarly, a study from Denmark investigated the prevalence of varicocele in school boys aged 10–19 years and found it to be 16.2% [17]. More recently, a military-based cross-sectional study included 7035 men from 6 European countries and reported a prevalence of 15.7%. In the same study, 0.2% had an isolated right-sided varicocele and 1.1% had bilateral varicocele [3, 18]. In another study that included 2061 military recruits aged from 19 to 34 years, the prevalence of varicocele was 24.2% [23]. Subsequently, large population-based studies reported a prevalence of 4–39% [19, 20, 22].

The variability in prevalence of varicocele may be related to the origin of the reports. Publications are from the Americas, Asia, Europe, and the Middle East. The prevalence varies according to the population characteristics, method of examination, and age at the time of the study [19, 21, 22]. Specifically, varicocele prevalence differs according to the age group with a less likelihood of finding varicocele in younger population compared to older population as it is related to changes in the valvular mechanism of venous system as men get older. As a result, this variability in the prevalence raises the importance of controlling the possible confounders when conducting an epidemiological study related to varicocele (age, BMI, height).

Prevalence of Varicocele in Pediatric and Adolescent Population

The prevalence of varicocele in the pediatric and adolescent population varies greatly according to the age group and the grade of varicocele at presentation. Moreover, this variation can be related to whether the studies were carried out in an epidemiologic fashion (school screening) or in clinical settings (patients with varicocele in urology clinic) [24]. Varicoceles are seldom seen in boys under 10 years old, but a noticeable increase in the prevalence at puberty was demonstrated by epidemiological studies [1, 24]. This rise in prevalence in post-pubertal boys has been linked to

the venous incompetence that occurs during testicular development [1].

One of the earliest studies on varicocele prevalence was published in 1960 by Horner. He investigated 1211 English boys and found no varicoceles in boys aged <11 years, but a prevalence of 15.9% in boys aged 11–16 years [25–27]. Oster studied a group of boys from Denmark and noticed that no varicoceles were found in boys with an age range of 6–9 years, but a steep increase in prevalence was detected in boys aged 10–19 years (16.2%) [17]. More recently, Akaby et al. assessed varicocele in 4052 Turkish boys with an age range of 2–19 years [26]. They reported an overall prevalence of 7.2%. The prevalence was <1% in boys aged 2–10 years and 11% in those aged 11–19 years. They found that 10.8% had bilateral varicocele and a right-sided varicocele was noticed in one boy. A Doppler study from Germany was performed in 2756 children and 2008 adolescents. The study revealed a prevalence of varicocele of 18% in children and 42.7% in adolescents [20].

Kumanov et al. reported on the prevalence of varicocele in 6200 boys between 0 and 19 years and found a 4.1% overall prevalence with a prevalence of 7.9% in boys with an age range of 10–19 years [28]. In this study, they found that height, age, body mass index (BMI), penile circumference, and penile length were factors implicated in the development of varicocele. The largest study to date was conducted in Israel on 1.3 million adolescent males aged between 16.5 and 19.9 years (mean age of 17.5 years) [19]. They found that the prevalence of varicocele was 1.6–4.6% according to the birth group and BMI. Furthermore, they suggested that there is an association between height and varicocele development possibly related to "nutcracker" phenomenon.

In the above mentioned epidemiologic studies, grade 1 was noted to be the most frequent grade [24]. On the other hand, urology clinic-based studies found that it is not common for adolescents to present to the clinic with a grade 1 varicocele (0–15%), but they mostly come with a grade 3 varicocele (68%) [24, 29–32]. This variation in grade distribution comparing population-based studies and clinic-based studies could be related to referral bias [24].

Age-Related Increase in Prevalence

There appears to be an association between aging and varicocele in some reports. In a study of 504 healthy adults 30–89 years old with a mean age of 54.7 years, 34.7% of the men were found to have varicocele [33]. The authors reported that the varicocele prevalence increases by 10% for each decade of life until it reaches 75% above the eighth decade [23, 33]. The trend toward increasing prevalence of varicocele and its relation with age was as follows: 18% at 30–39 years old, 24% at 40–49 years old, 33% at 50–59 years old, 42% at 60–69 years old, 53% at 70–79 years old, and 75% at 80–89 years old [1, 33]. A recent study by Liu et al. showed a similar trend. Varicocele prevalence in that study was 4.54% in the 21–29 years old cohort, 5% in the 30–39 years old cohort, and 6.14% in the 40–49 years old cohort [22]. These studies suggest that age-related changes affect the one-way mechanism of internal spermatic vein valves which results in their incompetence and the development of varicocele [33]. On the contrary, other studies did not establish an association between increasing age and varicocele [34, 35]. Canales et al. demonstrated that the varicocele prevalence in older men was 42% greater than younger patients but without an increase in varicocele prevalence with age and the possible reason being that most of the men in their study were older than 50 years [34].

Prevalence of Varicocele in Infertile Men

The early description of varicocelectomy indicates that the surgery was originally (in the late nineteenth century) performed for pain management primarily. The relationship between varicocele and infertility was not strongly established until the work of Tulloch in 1952 [36]. He performed a varicocelectomy in a patient with bilateral varicocele and azoospermia and the patient

experienced an increase in sperm count and a pregnancy occurred after the surgery [36, 37]. Thereafter, several studies were published on varicocele and its effect on fertility.

There is a considerable difference in varicocele prevalence between fertile and infertile men. Although varicocele may have an effect on fertility potential via several mechanisms, it is imperative to emphasize that not all varicocele patients are infertile, but the condition is encountered more commonly in men presenting to the infertility clinic [38]. Two studies on the association between varicocele and infertility found that 80% of adults with varicocele are able to father children [39, 40]. In men presenting for infertility evaluation, the prevalence of varicocele is in the range of 30–40%, making it the most frequent abnormal physical finding in infertility clinics [35].

The World Health Organization (WHO) evaluated the effect of varicocele on fertility parameters in 24 countries over a 12-month period [41]. 9034 men were recruited to evaluate physical findings and semen characteristics. They found that 25.4% of the adult male with abnormal semen parameters had a varicocele. On the other hand, they reported that the varicocele prevalence in adult male with normal semen parameters was 11.7% [41].

Gorelick and Goldstein investigated the varicocele prevalence in 1001 infertile adult males, and they noted that 35% with primary infertility and up to 81% with secondary infertility had varicocele [42]. Likewise, Witt and Lipshultz reported the findings of 2989 infertile adult males and demonstrated that varicocele prevalence is higher in adult male with secondary infertility (69%) compared to men with primary infertility (50%) [43]. The conclusion from both studies is that varicocele is a "progressive" disease resulting in a time-dependent decline in fertility potential. In contrast, Jarow et al. detected no difference in varicocele prevalence among 2188 adult males with primary and secondary infertility (45% and 44%, respectively) [44].

The observed discrepancies in the reported prevalence of varicoceles in the general and infertile male population may be due to lack of direct comparison between the two groups, the age-related increase in varicocele prevalence, sample size, referral bias (epidemiological studies vs. clinic-based studies), lack of proper control of confounding variables (e.g., BMI, hereditary factors, age, semen characteristics, associated venous insufficiency), and the variability in measures used for diagnosis (physical exam vs. scrotal ultrasound) that may differ from study to study depending on the physician expertise in doing the physical exam [1, 33, 45]. It is important to address the abovementioned limitation in the studies related to varicocele prevalence by accurately selecting the study population, standardizing the diagnostic measure, and controlling for any source of bias.

Hereditary Factors of Varicocele

From the few studies that have been published on the topic, there appears to be a hereditary factor involved in varicocele development. In 1992, Ziv et al. found no familial occurrence in the families with a member affected by varicocele and no association between the human leukocyte antigen (HLA) and varicocele [46, 47]. Raman et al. examined 44 patients with varicocele who agreed to include their 62 first-degree relatives (fathers, brothers, sons) [48]. They reported that 56.5% of the first-degree family members of varicocele patients had a palpable varicocele on physical examination, a prevalence 8-fold higher than a control group that included men who presented for vasectomy reversal (56.5 vs. 6.8%, $P < 0.0001$) [48]. They found fewer grade 3 varicoceles in the first-degree relatives compared to the patients, but it was not statistically significant. Importantly, in the same study, neither the grade of varicocele nor the presence of bilateral varicoceles was predictive of inheritance in the first-degree family members [48]. Moreover, they could not demonstrate a trend in the inheritance in a specific subgroup among the first-degree relatives (fathers, brothers, sons) but they found >70% of varicocele patients' brothers were affected by varicocele [48].

Subsequent studies have shown similar trends. Mohammadali Beigi et al. evaluated 131 brothers

of varicocele patients and found that the varicocele prevalence was 4.5-fold higher compared to a control group (45.8% vs. 10%, $P < 0.001$) [47]. Their results were in concordance with Raman et al. in that they also found that there was no relationship between varicocele grade and bilaterality and varicocele prevalence in the first-degree relatives [47]. Furthermore, Mokhtari et al. performed a prospective study of 62 patients known for varicocele, 88 first-degree relatives, and 100 controls (healthy men referred for kidney donation) [49]. They showed that 45.4% of the first-degree relatives of varicocele patients had a palpable varicocele which was greater than the control group (45.4% vs. 11%, $P < 0.001$) [49]. They also found that 50% of the brothers of varicocele patients had a palpable varicocele.

More recently, Gökçe et al. studied the varicocele prevalence among first-degree relatives of patients with a known varicocele [50]. They concluded that 34% of the first-degree relatives had a palpable varicocele which was 3-fold higher than the control group ($P < 0.005$) [50]. Of the first-degree relatives, 36.2% of brothers and 21.1% of fathers had palpable varicocele on physical exam [50].

On the basis of the previously mentioned studies, there appears to be an increase risk in the occurrence of varicocele in relatives of patients with varicocele. Nonetheless, there is no clear recommendation on counseling the relatives of varicocele patients [48, 49]. Additional studies with larger samples are needed to demonstrate the genetic factors that specifically associated with the increased varicocele prevalence among family members.

Varicocele Prevalence and Associated Conditions

Venous Insufficiency

A relationship between varicoceles and lower extremities varicose veins has been reported. One of the earliest studies that addressed this association was performed in 1986 by Dennison and Tibbs [51]. They concluded that varicocele and varicose veins share the same mechanism (reversed flow phenomenon). Further studies were done to clearly investigate the association between varicocele and venous insufficiency. In addition, patients with varicocele, particularly when bilateral, are at increased risk of an underlying systemic venous abnormality [52].

Ciaccio et al. reported their findings in 42 patients with varicocele, and they found that 85.7% of those patients had valvular incompetence in saphenofemoral junction [53]. Bolcal et al. conducted a larger multicenter study of 1500 patients with lower extremities venous insufficiency and they found 46% of them had varicocele [54]. They also showed that in patients with varicose veins, the following characteristics were potential risk factors for concomitant varicocele: high body mass index, positive family history, standing occupation, smoking, longer duration of symptoms, grade of venous reflux in the saphenofemoral junction, and constipation [54]. They recommended to counsel any patient with varicose veins about the risk of varicocele and infertility. Furthermore, Yasim et al. evaluated 100 patients presenting with varicose veins requiring surgery and observed that 72% had a clinical varicocele, many being grade 3 [55].

The largest study on varicose veins to date is a population-based nationwide study that included 2727 patients with varicose veins from the National Health Insurance Research Database in Taiwan and 10,908 randomly selected controls without varicose veins [56]. The authors reported the varicocele prevalence in patients with varicose veins compared to controls (1.3% vs. 0.3%, respectively, $P < 0.001$) [56]. They suggested a relationship between varicocele and varicose veins which was higher in patients <50 years old.

On the contrary, Yazici et al. demonstrated no statistical association between varicose veins and varicocele after studying 100 patients with varicocele [57]. They also stated that varicocele might not be attributed to systemic vascular insufficiency [57].

The association between cardiovascular comorbidities and varicocele has been explored by Kiliç et al. in their study of 52 varicocele patients aged between 14 and 50 years [58]. They demonstrated that the varicose veins prevalence was greater in

patients with varicocele compared to a control without varicocele and more importantly that varicocele is not associated with cardiovascular risk factors in patients <50 years old [58].

Finally, it has been suggested that varicose veins and varicocele share the same pathological and molecular mechanism. Valvular incompetence with decreased venous return leads to blood stasis which induces tissue hypoxia and upregulates the expression of hypoxia-inducible factor-1alpha (HIF-1a) and Bcl-2 (anti-apoptosis protein) in both varicose veins and varicocele [59]. Moreover, it has been found that Bcl-2 plays a protective role in the state of tissue hypoxia with associated apoptosis and contributes to the dilated veins seen in both varicose veins and varicocele [59].

Body Mass Index (BMI)

Studies on the relationship between BMI and varicocele prevalence report inconsistent findings. Several reports have shown an inverse relationship between BMI and varicocele, whereas other studies have not.

The first study that examined this association was conducted by Smith in 1957. This study evaluated 840 men with varicocele and found that these men were taller and heavier compared to a control group without varicocele [60, 22]. Delaney et al. (2004) examined the data of 43 adolescent boys (age range: 11–19 years) and demonstrated that patients with varicocele were heavier and taller compared to a control group, but BMI did not differ significantly between the two groups [61]. Kiliç et al. showed similar results in their study of 52 patients with varicocele aged between 14 and 50 years [58]. Few other studies have showed that varicocele patients have distinctly lower BMI compared to patients without varicocele [62, 63].

Most studies have demonstrated an inverse relationship between BMI and prevalence of varicocele. Nielsen et al. reported their results of 2106 men evaluated for erectile dysfunction or infertility and showed that the prevalence of varicocele decreases as BMI increases [35]. Subsequent published studies showed a similar association [23, 64–68]. It has been proposed in some of these studies that the reason for this inverse relationship between BMI and prevalence of varicocele could be due to the difficulty in detecting varicocele in obese patients due to a thicker spermatic cord and presence of adipose tissue in the scrotum and inguinal area [1, 22, 35, 64]. Another possible reason is that the "nutcracker" phenomenon is decreased in obese patients because the intra-abdominal adipose tissue provides a cushion that decreases the compression of left renal vein between the aorta and superior mesenteric artery [22, 35, 64].

The largest study on the relationship between BMI and prevalence of varicocele was conducted by Rais et al. These authors reviewed data on 1.3 million adolescent males with an age range between 16.5 and 19.9 years [19]. Their study revealed a strong association between BMI and prevalence of varicocele independent of confounders [19]. In the same study, they rejected the claims of the previously mentioned reports regarding the reasons for the inverse relationship between BMI and prevalence of varicocele (difficult physical exam in obese men and decreased "nutcracker" phenomenon due to adipose tissue). They provided a critical assessment of previous reports. First, prior studies were not population-based as they included a small sample of infertile males [19]. Second, none of the previously mentioned studies controlled for the possible confounders. Third, they commented that a study by Walter et al. [69] found that obese men have a lower prevalence of ultrasound-detected varicocele than normal weight men suggesting that a difficult physical exam should not be perceived as a reason for the lower prevalence of varicocele in obese men [19]. Fourth, they suggest that further verifications are needed to examine the potential effect of adipose tissue on the "nutcracker" phenomenon. This could be done by ultrasound examination of the left renal vein, measurement of visceral fat and assessment of waist-hip circumference [19].

Table 8.1 Summary of the important studies in varicocele epidemiology

Study	Population	Number of patients	Findings
Horner (1960)	School boys	1211	No varicocele in boys <11 years old, varicocele found in boys aged 11–16 years = 16%
Clarke (1966)	Healthy men from Marine Corps Reserves	275	Varicocele prevalence = 8%
Steeno et al. (1976)	School boys & college students	4067	Varicocele prevalence = 14.7%
WHO (1992)	Men from infertility clinics from 24 countries	9034	25.4% with abnormal semen parameters had varicocele, varicocele prevalence = 11.7% in men with normal semen parameters
Gorelick & Goldstein (1993)	Infertile adult male	1001	Varicocele prevalence = 35% in men with primary infertility, 81% in men with secondary infertility
Jarow et al. (1996)	Infertile men in 3 infertility centers	2188	No difference in varicocele prevalence among men with primary & secondary infertility(44% & 45%)
Akaby et al. (2000)	Day care & school boys (2–19) years old	4052	Varicocele prevalence = 7.2%, <1% in boys (2–10) years old
Delaney et al. (2004)	Adolescent men with varicocele	43	No significant in BMI
Nielsen et al. (2006)	Males evaluated for erectile dysfunction or infertility	2106	Prevalence of varicocele decreases as BMI increases
Bolcal et al. (2006)	Young men with lower limb venous Insufficiency	1500	Varicoceles were found in 46% of the patients
Kumanov et al. (2008)	Boys 0–19 years old	6200	Varicocele prevalence = 4.1%, height, age, BMI, penile length were risk factors
Soylemez et al. (2012)	Military recruits, Turkey	2061	Varicocele prevalence = 24.2% (prevalence decreases as BMI increases)
Rais et al. (2013)	Military recruits, Israel	1.3 Million	Largest study, Varicocele prevalence = 1.6–4.6%, BMI is inversely related to varicocele
Lai et al. (2015)	Population-based, men with varicose veins compared to controls	2727	Varicocele prevalence in varicose veins vs. controls (1.3% & 0.3%, <0.001)
Damsgaard et al. (2016)	Military men, 6 European countries	7035	Varicocele prevalence = 15.7%

Conclusions

In summary, there is variability in the reported prevalence of varicocele across different studies. The variability may be related to the origins of the reports, the method used to detect varicocele, and the population characteristics. Moreover, there is variation in the grade distribution among pediatric and adolescent population. Varicocele is a "progressive" disease resulting in a time-dependent decline in fertility potential. Furthermore, there appears to be an increased risk in the occurrence of varicocele in relatives of patients with varicocele, but there is no clear recommendation on counseling the relatives of varicocele patients. Finally, several reports have shown an inverse relationship between varicocele and BMI. Additional larger studies are needed to completely understand varicocele epidemiology (Table 8.1).

Review Criteria

An extensive search was done including articles from 1952 to 2017. The PubMed and MEDLINE search terms included "varicocele," "infertility," and "epidemiology." The main focus was on studies discussing clinical varicoceles epidemiology and their relationship to male infertility.

Multiple Choice Questions and Answers

1. The prevalence of varicocele in men with primary infertility is:
   (a) 20%.
   (b) 50%.
   (c) **35%.**
   (d) 70%.
2. What is the most widely known mechanism that accounts for impaired spermatogenesis seen with varicocele?
   (a) **Scrotal hyperthermia.**
   (b) Angles of insertion of the left internal spermatic veins.
   (c) The reflux of renal and adrenal metabolites.
   (d) Norepinephrine.
3. The largest study to date that was conducted in Israel on 1.3 million adolescent males found the prevalence of varicocele to be:
   (a) 5.1–7%.
   (b) **1.6–4.6%.**
   (c) 9–11%.
   (d) 13–15%.
4. All of the following regarding hereditary factors in varicocele are correct EXCEPT:
   (a) First-degree family members of patients with varicocele had 4–8-fold higher prevalence compared to a control group.
   (b) **There is an association between HLA and varicocele.**
   (c) There is no association between varicocele grade and bilaterality and varicocele prevalence in the first-degree relatives.
   (d) There is no clear recommendation on counseling the relatives of patients with varicocele.
5. Dilated veins seen in both varicose veins and varicocele has been linked to:
   (a) HIF-1a.
   (b) Prostaglandins.
   (c) IL-23.
   (d) **Bcl-2.**

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Association Between Varicocele and Infertility

9

Daniel Lybbert and Nicholas N. Tadros

Key Points

- The detrimental effects of varicocele are multifactorial and effect many cells and associated physiologic processes that contribute to the production, maturation, and transport of viable sperm.
- Varicocele has been associated with an increase in intrascrotal temperature, accumulation of reactive oxygen species, DNA fragmentation, and alteration of the cell membrane and cytoskeleton.
- Varicocele impacts Leydig cells, resulting in decreased serum and intratesticular testosterone levels. Varicocelectomy has also demonstrated an improvement in men who also have hypogonadism.
- Inhibin B, a marker of Sertoli cell function, is found to be decreased in the setting of varicocele and is likewise improved after varicocelectomy.
- The transport of sperm through the epididymis is accelerated in the setting of clinical varicocele, which results in inadequate maturation and decreased viability of the sperm.

Correlation Between Varicocele and Infertility

Infertility is attributed to a male factor in 50% of couples being treated for infertility, either alone or in combination with a female factor. Varicocele, a pathological dilation of the scrotal veins, is thought to be the cause of infertility in up to 41% of male factor infertility [1] and is the most common cause of secondary infertility [2]. Although the pathophysiology of varicocele is not well understood, there is a clear effect on semen analyses and resulting infertility. A meta-analysis by Agarwal et al. revealed that men with varicocele have reduced sperm counts, impaired motility, and abnormal morphology when compared to men without varicocele [3]. However, 80% of men with some degree of varicocele lack any evidence of infertility [4]. Many studies, though, have clearly demonstrated that treatment of clinical varicocele in symptomatic men improves semen parameters and restores fertility. A large meta-analysis of 17 such studies found that after varicocelectomy, semen analysis had a mean increase in sperm density of 9.7 million/mL, a 9.9% motility increase, and a WHO sperm morphology improvement of 3% [1]. Other studies have also shown improvements in additional parameters such as decreased DNA fragmentation [5], decreased levels of oxidative stress [6], and improved sperm penetration assays [7].

D. Lybbert · N. N. Tadros (✉)
Department of Surgery, Division of Urology, Southern Illinois University, Springfield, IL, USA
e-mail: ntadros78@siumed.edu

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Along with these improvements in semen analysis, men being treated for varicocele are more likely to have a resultant pregnancy. Several retrospective studies have observed pregnancy rates anywhere from 30% to 50% following varicocelectomy [8–10]. A meta-analysis of five different studies found that varicocelectomy resulted in a pregnancy odds ratio of 2.87 compared to no treatment and a number needed to treat of 5.7 [11].

Even in azoospermic men, varicocelectomy has shown to be of benefit, with some men being able to go on to have natural pregnancies with their partners [12, 13]. In a study of 22 men with azoospermia and 56 men with oligoasthenozoospermia, Matthews et al. found significant improvements in fertility after varicocelectomy. 12 of 22 azoospermic men were found to have motile sperm and sperm counts averaging 2.2×10^6. Subsequently, three of those men went on to achieve a pregnancy, including two who were able to conceive naturally without assistance [14].

Effect of Varicocele on Testicular Function

The deleterious effects of a varicocele can impact multiple testicular cell types, resulting in an interdependent, multifactorial decline in fertility. Although we are still unsure exactly how a varicocele can have such deleterious effects on fertility in some men, while others remain asymptomatic, much has been discovered on the subject that has allowed us to better treat men with clinical varicocele.

Believed by many to be the greatest contributing factor to infertility is hyperthermia. The countercurrent flow provided by the venous plexus in the spermatic cords allows for the testicles to remain at a temperature 1–2 °C below that of body temperature [15]. Multiple animal studies have shown that in hyperthermic environments, germ cells undergo apoptosis secondary to oxidative stress [16, 17]. Interestingly, hyperthermic conditions within the scrotum have shown to contribute to infertility independently of the grade of varicocele [18].

Other theories suggest that reactive oxygen species and other metabolic wastes accumulate in the testicle which may cause damage to the germ cells and supporting cells. This has been recorded in multiple studies that have found increased levels of reactive oxygen species within semen [19–21]. This may be a result of hemostasis, as the blood pools in the dilated pampiniform plexus veins and prevents proper evacuation of waste products. A study by Ozbeck et al. found renal and adrenal metabolites within the venous plexus of the testicles, suggesting that incompetent valves allow for the reflux of blood which further contributes to dilation of the venous collecting system and hemostasis [22].

What we do know for sure, however, is that there are many contributing factors that lead to infertility as a result of varicocele. There is no one thing that is the all-cause factor, which is what makes treating men with infertility such a challenge.

There are two important functioning cell types in the testicles, the Leydig cells and the Sertoli cells, which are responsible for testosterone production and spermatocyte nurturing, respectively. Both cell types are needed for the production of healthy spermatocytes. Both cell types are negatively impacted by the effects of clinically significant varicocele. The germ cells from which spermatocytes are derived can also be directly affected by varicoceles.

The testosterone produced by the Leydig cells is essential for the development of viable sperm and can be adversely affected by a clinically significant varicocele, and at least four important aspects of spermatogenesis can be affected by injury to the Leydig cells (see Fig. 9.1):

- Meiosis
  - The process of meiosis cannot be carried out to completion without testosterone signaling. Testosterone alone is responsible for the expression and modification of many proteins involved in oxidative metabolism, DNA repair, RNA processing, apoptosis, and meiotic division. Without testosterone signaling, meiosis is halted [23].

- Blood-Testis Barrier Maintenance
  - The blood-testis barrier is constantly being remodeled as spermatocytes develop and detach from the basement membrane of the seminiferous tubules [24]. The production of the proteins responsible for formation of the tight junctions that make up the blood-testis barrier is upregulated by testosterone [25]. When testosterone levels decrease, the remodeling of the blood-testis barrier slows, which in turn slows the process of spermatogenesis [23].
- Sertoli-Spermatid Adhesion
  - Testosterone signaling also regulates the expression of adhesion complexes between the Sertoli cells and developing spermatids. In the absence of testosterone signaling, spermatids detach themselves from the Sertoli cells before they develop sufficiently to survive independently [23].
- Sperm Release
  - Interestingly, testosterone signaling also plays a role in the release of mature sperm from the Sertoli cells. Testosterone, in conjunction with FSH, signals changes in adhesion proteins to allow the dissociation of sperm from the Sertoli cells. Without this dissociation, the mature sperm cells eventually become phagocytized and destroyed by the Sertoli cells [23].

Consequently, we observe that once serum testosterone levels decrease significantly, sperm counts also diminish. Zirkin et al. studied the effects of androgens on sperm production in rats and found that once intratesticular testosterone

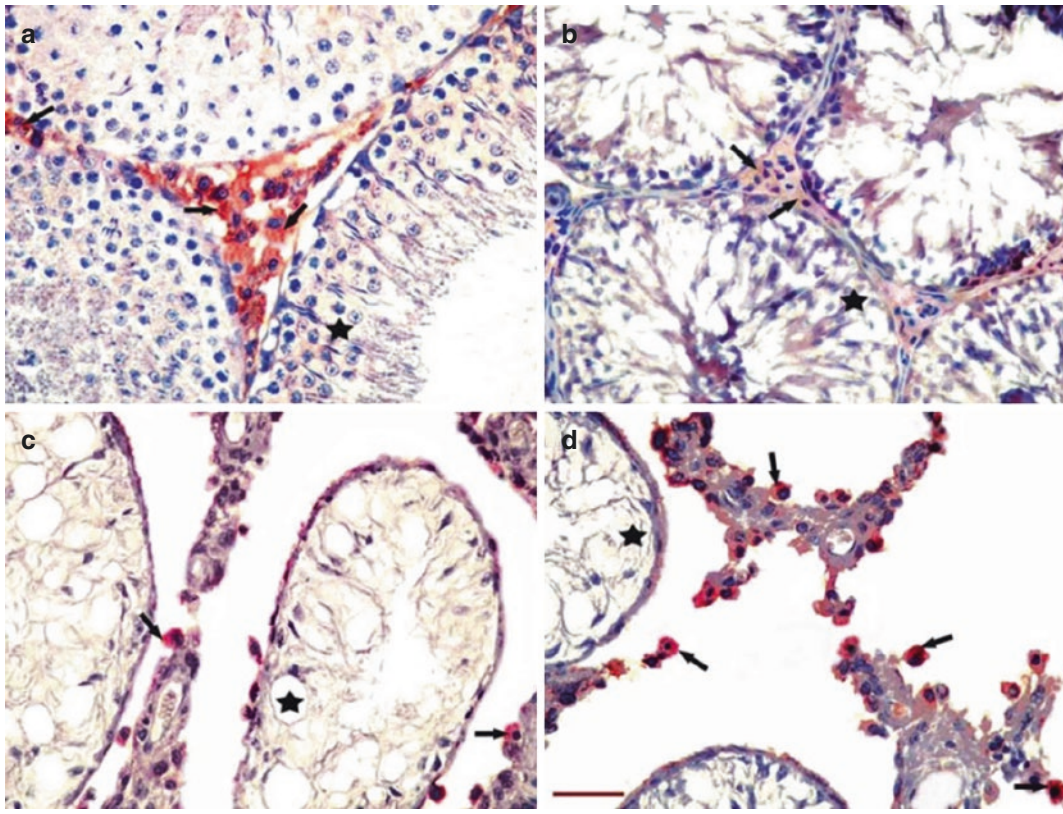

Fig. 9.1 Immunohistochemical staining of testosterone-positive Leydig cells in scrotal hyperthermia applied to different groups. (**a**) Control, (**b**) 1 day after scrotal hyperthermia, (**c**) 14 days after scrotal hyperthermia, (**d**) 35 days after scrotal hyperthermia. *Arrow*, immune-positive of Leydig cells. *Asterisks*, germinal epithelium. Scale bar = 50 μm. (Reprinted from Aktas and Kanter [46]. With permission from Springer Nature)

levels fell below a predefined threshold, there was a significant decrease in sperm production [26]. Observation of Leydig cells in hyperthermic testicles has demonstrated cellular damage in the form of atrophy and cytoplasmic vacuolization, resulting in decreased numbers of Leydig cells [27]. Although there has been conflicting data as to whether or not varicocele is a cause of hypogonadism, a large study by the WHO of over 9000 men demonstrated that men over the age of 30 with varicocele had significantly lower serum testosterone levels than men under the age of 30 with varicocele, a trend not seen in men without varicocele. This suggests that varicocele can impact testosterone levels over time [28]. There is also an increasing body of literature that shows that after men with clinical varicocele undergo varicocelectomy, there is a large increase in serum testosterone levels and in testicular size [29–31]. One study in particular compared 200 men with varicocele and hypogonadism presenting to an infertility clinic, with half undergoing varicocelectomy and the other half choosing assisted reproductive techniques (ART) instead. Seventy-eight percent of men in the varicocelectomy group had a normalization of testosterone levels, while only 16% of the ART group demonstrated normalization, suggesting that varicoceles have a direct impact on Leydig cell function and testosterone production [32].

Sertoli cells, responsible for the direct support of developing germ cells, are also impacted by the environment created by clinical varicocele. Sertoli cells maintain the blood-testis barrier, coordinate all transference of spermatogonia through the blood-testis barrier, nourish and regulate immunomodulation of developing spermatogonia, and consume excess components of developed spermatozoa. Fertility is impacted when any one of these processes are hindered by the effects of a clinical varicocele.

Sertoli cell function can be monitored by measuring the level of Inhibin B circulating in the serum. Inhibin B, produced in great amounts by the Sertoli cells, acts at the anterior pituitary to inhibit the production of FSH. It is often decreased in infertile patients with a testicular cause of infertility [33]. This marker of Sertoli cell function is also found to return to normal levels after successful varicocelectomy and improvement in fertility [34]. Similar findings with transferrin- and androgen-binding protein have also been associated with damage to the Sertoli cells and subsequent infertility [35]. Just like with inhibin B, transferrin- and androgen-binding protein levels seem to improve with successful varicocelectomy that also results in improved fertility [36].

But what exactly is happening to Sertoli cells that prevent them from carrying out their functions to support the germ cells? Studies on rats have shown that Sertoli cells in hyperthermic environments have changes to their lipid membrane composition [37]. Changes have also been observed in the cytoskeleton and cell binding proteins of the Sertoli cells in the setting of low testosterone [23]. The cytoskeleton, protein composition, and lipid membrane structure are all critical to the Sertoli cells' ability to phagocytize and modify the developing spermatids. Without the ability to consume apoptotic bodies and residual membrane from the developing spermatids, the resulting sperm in the semen are deformed and have decreased motility. Changes to the cytoskeleton and lipid membrane also impact the ability of the Sertoli cells to modify the blood-testis barrier. Without the ability to modify this barrier and allow the developing spermatocytes to pass through, their development is halted and sperm counts decrease [38].

Effect of Varicocele on Epididymal Function

The adverse effects of varicoceles also extend to the epididymis. The epididymis does more than just temporarily store developed sperm for eventual ejaculation. Sperm in the epididymis undergo the final phase of maturation that improves their motility and allows them to penetrate and fertilize the egg. The health of the epididymis can be monitored by measuring seminal alpha-glucosidase. Alpha-glucosidase is an enzyme produced by the epididymis that is found to be low in men diagnosed with infertility secondary to epididymal obstruction or dysfunction [39, 40].

One study evaluated 60 men with varicocele who were found to have seminal neutral alpha-glucosidase levels, 56% lower than the control group of 30 men without varicocele [41]. In another study of 50 men who underwent varicocele embolization, there was a significant increase in alpha-glucosidase activity from a mean of 61.7 pre-procedure to 84.7 units post-procedure [42]. The timing of travel through the epididymis is important, and the effects of varicocele have been found to impair that timing and the subsequent functions that occur within. Fernandez et al. used rats to demonstrate the effects of increased transit times of sperm through the epididymis. By using diethylstilbestrol, sperm transit time through the epididymis was accelerated without effecting sperm production in the testes. Compared to the control and another group where guanethidine was used to slow sperm transit time, the rats with accelerated transit time had sperm with impaired motility and decreased sperm counts in the semen, resulting in decreased fertility. This same study also demonstrated the effect of androgens on epididymal transit times. The group exposed to diethylstilbestrol was also found to have low serum testosterone levels because of its estrogenic effects. When those rats were provided supplemental testosterone, viability of the sperm and transit time improved to that of the control group [43]. Androgen withdrawal via castration demonstrated similar results of accelerated epididymal transport time and subsequent infertility in rats without the addition of estrogenic compounds [44]. Both scenarios showed that both low androgens levels and accelerated epididymal transit times resulted in less viable sperm.

Lehtihet et al. studied the effects of large grade 3 varicoceles and the results after repair. Their studies suggested that mass effect from the varicocele compressing the epididymis may also accelerate the transit time through the epididymis, contributing to subsequent infertility [42].

The amount of time that sperm can remain functional after storage, or sperm storage time viability, in the epididymis may also be affected by the electrolyte concentrations within the epididymis. The epithelial cells within the epididymis regulate the environment within the lumen by way of transport proteins that allow for transference of water and ions. When exposed to higher temperatures, such as in the presence of a varicocele, this delicate transport system and the balance of ions within the lumen of the epididymis are compromised. A study from Cornell showed that in hyperthermic conditions, the epithelium of the caudal epididymis had impaired ion and water transport, resulting in more water, higher concentrations of Na^+ and Cl^-, and decreased concentrations of K^+ within the lumen. Subsequently, the sperm from these altered specimens had a decreased storage time viability. The authors of that study reference multiple other studies which demonstrate that increased temperatures alone do not impact sperm storage time viability, suggesting that the environment within the lumen of the epididymis is what sustains the sperm during storage. Whether or not the concentration of these particular ions was the cause of impaired sperm viability or some other pathway regulated by transport mechanisms that were not measured is unclear, it demonstrates that the environment of the epididymal lumen is subject to change in conditions such as varicocele and that this can impact fertility [45].

Conclusion

Although many questions remain regarding the pathophysiology of varicoceles and their effects on fertility, much has been discovered, allowing us to both better understand and better treat men with varicocele. Studies on how hyperthermia in particular affects the function of the testicles and epididymis have expanded our understanding, but a clinically significant varicocele does more than just increase intrascrotal temperatures. Varicocele has been shown to increase the concentration of reactive oxygen species and metabolic waste products, decrease the oxygen content, obstruct blood flow, and allow for reflux of active chemicals that all may play a part in infertility. More work is needed to better understand how varicoceles impact testicular and epididymal function and subsequent spermatogenesis.

Review Criteria

An extensive search of research and literature on the subject of varicocele and its pathophysiology at the level of individual cell types within the testicle and epididymis was done using search engines including Google Scholar, PubMed, MEDLINE, ScienceDirect, and ClinicalKey. The search was conducted between February 2018 and August 2018. Literature and data reviewed was found using the following keywords: "varicocele," "varicocelectomy," "Leydig cell," "Sertoli cell," "germ cell," "epididymis," "infertility," "scrotal hyperthermia," "reactive oxygen species," "inhibin B," "testosterone," and "semen parameters." Literature in languages other than English were considered. Data published solely from meeting proceedings, lectures, websites, or books were not included.

Multiple Choice Questions and Answers

1. Which of the following is *false*?
   (a) Testosterone signaling regulates the expression of adhesion complexes between the Sertoli cells and developing spermatids.
   (b) **The production of the proteins responsible for formation of the tight junctions that make up the blood-testis barrier is inhibited by testosterone (it is upregulated).**
   (c) Testosterone, in conjunction with FSH, signals changes in adhesion proteins to allow the dissociation of sperm from the Sertoli cells.
   (d) Testosterone alone is responsible for the expression and modification of many proteins involved in oxidative metabolism, DNA repair, RNA processing, apoptosis, and meiotic division.
2. Which of the following effects of varicocele is believed to have the greatest negative impact on fertility?
   (a) Reflux of renal and adrenal metabolites into the pampiniform plexus
   (b) **Elevated intrascrotal temperature**
   (c) Decreased transit time through the epididymis
   (d) Accelerated remodeling of the blood-testis barrier
3. Which of the following chemicals can be measured to indicate Sertoli cell function?
   (a) Alpha-glucosidase
   (b) Testosterone
   (c) Diethylstilbestrol
   (d) **Inhibin B**
4. Which of the following is true?
   (a) 80% of men with varicocele will have some degree of impaired fertility.
   (b) Men with varicocele and associated infertility have been found to have elevated alpha-glucosidase levels and increased epididymal transit times.
   (c) **Testosterone signaling regulates the remodeling of the blood-testis barrier, an important factor in progression of spermatogenesis.**
   (d) Varicocelectomy not only improves fertility but has been shown to reverse hypogonadotropic-hypogonadism.
5. Which of the following men would most likely benefit from varicocelectomy for infertility?
   (a) **32-year-old male with unilateral grade 3 varicocele and normal serum testosterone levels, normal FSH, and LH**
   (b) 27-year-old male with bilateral varicocele diagnosed on ultrasound and testosterone levels on the low end of normal, normal FSH, and LH
   (c) 47-year-old male with visible varicocele on the left and low-grade varicocele on the right, low testosterone, low FSH, and low LH
   (d) 34-year-old male with bilateral varicocele palpable upon Valsalva with elevated testosterone, low FSH, and low LH

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Varicocele Clinical Diagnosis and Grading

10

Mohamed Arafa and Haitham Elbardisi

Key Points

- Proper history taking and physical examination are the cornerstone for clinical diagnosis of varicocele.
- Varicocele is generally *asymptomatic*.
- Presence of varicocele in acute scrotal pain is a mere coincidence.
- Proper physical examination and grading of varicocele needs experienced male infertility specialist.
- Only clinically palpable varicocele associated with manifestations should be treated.

Introduction

Varicocele is generally found in 15% of men and approximately 40% of infertile men. This high prevalence of varicocele in humans may be explained by scrotal venous congestion caused by the erect position of humans [1, 2].

Varicocele is usually asymptomatic. However, it may present with infertility, testicular pain, scrotal swelling, or hypogonadism or may be incidentally found during physical examination for army recruitment or athlete's evaluation. It is crucial to have a proper diagnosis of varicocele for the sake of proper management; otherwise varicocelectomy for improperly selected candidates may lead to worsening of orchialgia due to increased venous congestion. Unnecessary delay in treatment may result in case of missed diagnosis of clinical varicocele.

Varicocele is more on the left side due to anatomical and physiological differences between both sides leading to higher hydrostatic pressure in the left spermatic vein with consequent dilatation and reflux [3, 4]. However, an associated right-side varicocele is present in 30–80% of cases but is mostly subclinical. Isolated right-side varicocele is very rare and warrants further investigations to rule out retroperitoneal pathology [5].

There is consistency among all international societies, American Society for Reproductive Medicine (ASRM), American Urological Association (AUA), and European Association of Urology (EAU), about detection of varicocele being mainly through proper history taking, physical examination, and semen analysis. The only controversy in the diagnosis of varicocele is the use of the ultrasound which was only recom-

M. Arafa (✉)
Department of Andrology, Cairo University, Giza, Egypt

Department of Urology, Hamad Medical Corporation, Doha, Qatar

H. Elbardisi
Department of Urology, Hamad Medical Corporation, Doha, Qatar

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Table 10.1 Diagnosis of varicocele according to different international guidelines

	History	Physical exam	SFA	Ultrasound
EAU 2016 guidelines [6]	✓	✓	1. If normal 2. If abnormal	Clinical examination should be confirmed by ultrasound Investigation and color duplex analysis
ASRM 2014 Report on varicocele and infertility: A committee opinion [7]	✓	✓	2 SFA	Only if inconclusive physical examination
AUA 2010 best practice statement [8]	✓	✓	2 SFA	Only if inconclusive physical examination

mended by EAU as being mandatory for the diagnosis (Table 10.1).

The aim of the chapter is to provide a detailed algorithm for clinical diagnosis of varicocele and to provide physicians with a clear guide for varicocele diagnosis.

Clinical Presentations

Infertility

Varicocele is the most surgically correctable cause of male infertility presenting in 19–40% of men with primary infertility and up to 81% in males with secondary infertility [9]. In these cases, varicocele is usually an incidental finding during clinical evaluation for fertility. This was further confirmed by WHO report in 1992 stating the presence of clinical varicocele in 11.7% of infertile normozoospermic men and 25.4% of infertile men with abnormal semen parameters [10].

The effect of varicocele on semen quality has been extensively studied in the literature. It can affect all semen parameters including sperm count, total and progressive motility, normal morphology, and vitality, as well as sperm function tests with elevation of sperm DNA fragmentation and increase in seminal oxidative stress [11, 12].

The exact pathophysiology of varicocele in fertility is still not clear. The most accepted theories are testicular hyperthermia, accumulation of toxins within the testis, reflux of renal and adrenal metabolites, and hypoxia. All this will lead to increase in the oxidative stress in the testis with subsequent affection of the function of Sertoli as well as Leydig cells [13].

Surgical correction of clinically palpable varicocele generally improves semen parameters in infertile men.

Pain

Painful symptomatizing varicocele is typically characterized by dull aching pain or heaviness in the scrotum, testis, or rarely groin. It increases with prolonged standing or physical activity which may affect the patient's style of life especially in sports professionals or army recruits. It may be alleviated by lying down or elevation of the testes. The pain is more often related to the left testicle since varicocele is more predominant on left side but may rarely be bilateral in cases of bilateral varicocele [14].

The varicocele-induced pain is usually chronic in nature since the varicocele is found since puberty. Therefore, in cases with acute scrotal pain, incidental finding of varicocele should not hinder the search for other causes of acute scrotal pain, e.g., torsion, epididymitis, or trauma. Rarely, the varicocele pain may be acute in nature, but usually it is not severe [15].

The effect of varicocele on male fertility is well documented; however the pathogenesis of varicocele in chronic orchalgia is still not clear. Testicular congestion due to venous dilatation and poor venous drainage with consequent hyperthermia may be a cause. Also, testicular hypoxia, reflux of renal and suprarenal metabolites, and high levels of reactive oxygen species may play a role in testicular tissue damage and consequently pain [16].

An interesting question regarding painful varicocele is why is the pain present only in some and

not all men with varicocele? In other words, what are the predisposing factors for pain in varicocele patients? Chen and Chen in 2012 compared between normozoospermia men with varicocele with and without associated pain. They stated that lower body mass index (BMI) seems to be a risk factor for developing pain with varicocele. Also, higher scrotal temperature, higher retrograde flow in spermatic veins, and longer distance from renal hilum to scrotum are reported as other risk factors. There is no significant difference in age and grade of varicocele between both groups [17].

Proper history taking is the key for diagnosing varicocele pain. The history must include detailed information on the location of the pain (varicocele pain is usually localized to the testis), the character, duration, and severity of the pain as well as aggravating and alleviating factors. Ruling out other causes of chronic orchalgia is very important in the diagnosis of varicocele pain as testicular pain may be a manifestation of a much more serious condition than varicocele. Furthermore, offering surgical treatment for such patients may aggravate the pain. Therefore, the history questionnaire should include previous diagnosis of hernia or hernia repair (pain may be related to entrapment of pudendal nerve), urinary stone diseases (pain may be referred from ureteric stone), and testicular or groin trauma; history of inflammation, epididymitis, orchitis, or prostatitis; previous testicular torsion; history of scrotal swelling, testicular masses (malignant or benign), extratesticular scrotal masses, hydrocele, or spermatocele; and history of scrotal surgeries, vasectomy, hydrocelectomy, spermatocelectomy, scrotal exploration, or orchiopexy [9, 18, 19].

Scrotal Swelling

High grade varicocele may present with scrotal swelling with or without associated pain. This is usually accidentally discovered during self-examination or during physical examination for army recruitment or sports. Even though the ipsilateral side might look larger than the contralateral side, on clinical examination, testicular size is usually smaller on the ipsilateral side of varicocele [20].

Hypogonadism

Men presenting with symptoms suggestive of hypogonadism (decreased libido, erectile dysfunction, etc.) need to be thoroughly evaluated to detect possible correctable causes including examination for varicocele. Similar to its deleterious effect on spermatogenesis, varicocele may inhibit the endocrine function of the testis through multiple mechanisms; hyperthermia leads to Leydig cell atrophy, structural changes of Leydig cells, as well as decrease in enzymes involved in testosterone synthesis especially 17 alpha-hydroxyprogesterone aldolase which converts 17-hydroxyprogesterone to testosterone; therefore there is decrease in the Leydig cell mass and function [21–23].

Eighty percent of hypogonadal patients undergoing varicocelectomy had improvement in their testosterone level post-operatively. However, the primary indication for varicocelectomy in these studies was either pain or infertility [11].

Clinical Examination

Clinical examination is the mainstay of diagnosis of varicocele. Only clinically palpable varicoceles have been clearly associated with infertility.

While examining patient for varicocele, the physician must not skip general examination which may give leads to diagnosis and management of varicocele. General examination must be implemented to detect signs of hypogonadism such as sparse body and pubic hair and gynecomastia. It may also help in the differential diagnosis of testicular pain, the presence of abdominal scars of previous hernia repair or orchiopexy, scars of pelvic trauma, and tenderness in the loins or lower back.

How to Examine a Patient for Varicocele

Scrotal examination for varicocele is typically done in a quiet warm room to allow for psycho-

logical relaxation of the patient as well as relaxation of dartos and cremasteric muscles to facilitate inspection and palpation of varicocele. A cold atmosphere or patient's anxiety may limit the examination. Examination should be done with the patient in both the upright and recumbent positions. The patient should be exposed from his waist down enabling the physician to visualize the whole groin area.

Scrotal examination should be carried in a systematic way starting by inspection of the scrotal area for varicocele, scars, abscesses or sinuses, or any abnormality. This is followed by palpation of each testis individually usually starting with the normal side first. Examination is done bimanually to detect testicular size with careful palpation of the whole testis to detect any abnormality. Testicular size evaluation can be done using orchidometer for more accurate results. It has been reported that unilateral varicocele is usually associated with decreased testicular volume in the ipsilateral testicle and that the degree of this loss is proportionate with the clinical grade of varicocele being highest in large grade varicocele [24]. It is important to evaluate discrepancy in size and consistency between both testicles, to detect any testicular lesion, and to assess testicular tenderness (usually associated with orchitis). The epididymis is next to be examined to detect its presence, tenderness, irregularities, or masses. Then careful palpation of the vas deferens on each side is done to detect presence and irregularities.

Examination of the spermatic cord for varicocele is done by gently squeezing the neck of the scrotum between the thumb and index finger. It is done in the relaxed state at first. Usually, big varicocele is felt like a bag of worms above or behind the testicle. With smaller varicocele, the dilated veins could be felt along the course of the spermatic cord. The patient is then asked to perform Valsalva maneuver to detect reflux in the spermatic veins due to retrograde blood flow in the veins. Examination is then repeated with the patient in the recumbent position to detect emptying of the veins.

Clinical Grading of Varicocele

Clinical grading of varicocele is dependent on careful inspection and palpation of the scrotum in both relaxed state and with Valsalva. The most accepted and widely used classification is the Dubin and Amelar's [25]:

- *Grade I* varicocele is the varicocele that can be felt only with Valsalva maneuver.
- *Grade II* varicocele, dilated veins, can be felt during palpation without Valsalva but is not visible through scrotal skin.
- *Grade III* varicocele could be visualized through scrotal skin even with the patient in the relaxed state.

Subclinical varicocele cannot be detected by clinical examination but is only evident during radiological examination.

A new clinical grading system was recently proposed to refine and standardize varicocele grading more. However, this classification is not yet validated by clinical studies [26]. It states the following:

- *Grade 0 (subclinical)*: (i) Veins not palpable or visible, with or without Valsalva; (ii) no change in cord or testis upright vs supine.
- *Grade I (small)*: (i) Full veins when upright, collapse when supine; (ii) increased turgidity of veins with Valsalva; minimal or no impulse with Valsalva; (iii) firm testis upright, soft testis supine.
- *Grade II (medium)*: (i) Full tortuous veins upright; palpable but still invisible; (ii) increased turgidity of veins and distinct impulse with Valsalva; (iii) firm testis upright, soft testis supine.
- *Grade IIIa (large)*: (i) Easily visible through scrotal skin when standing upright; (ii) increased turgidity of veins and distinct impulse with Valsalva; (iii) firm testis upright, soft testis supine.
- *Grade IIIb (very large)*: (i) Veins fill entire ipsilateral hemiscrotum; (ii) increased turgid-

ity of veins and distinct impulse with Valsalva; (iii) firm testis upright, soft testis supine.
- *Grade IIIc (huge)*: (i) Veins fill entire scrotum, displacing contralateral testis; (ii) increased turgidity of veins and distinct impulse with Valsalva; (iii) firm testis upright, soft testis supine.
- *Subgrade p (pathologic)*: Varicocele of any grade that does not collapse in the supine position; retroperitoneal pathology must be ruled out.

Accuracy of Clinical Examination

The main limitation with clinical examination is its subjective nature with significant differences between physicians in the diagnosis and grading of varicocele [27]. This is also affected by the physician's experience. A study comparing two experienced infertility specialists and two unexperienced urologists found that false positive diagnosis of varicocele was found in up to 32% in unexperienced urologists compared to 24% in infertility specialists [27, 28]. There was even a discrepancy between the two infertility specialists where false positive diagnosis of varicocele was found in 11.5% and 24%. This was further confirmed by another study comparing clinical examination between ten clinicians. They found great inter-observer differences in diagnosis of varicocele as well as intra-observer differences with only one third of physicians being able to reproduce their results in subsequent days [29].

Clinical examination is also hindered by the body shape, anatomical variations of the scrotum, and varicocele grade. Obesity may make scrotal examination difficult. Also, small varicoceles are usually more difficult to palpate especially with unexperienced clinicians. Abnormal scrotal anatomy may obscure clinical examination of varicocele, e.g., hydrocele, tight scrotal sac, previous scrotal surgery, lipoma of the spermatic cord, or thickening of the scrotal wall as in lymphedema.

Compared to radiological modalities, including color Doppler ultrasound, venography, and scrotal thermography, clinical examination showed the least sensitivity in detection of varicocele. While venography has the best sensitivity reaching nearly 100%, its invasive nature hinders its routine use for clinical diagnosis of varicocele and is reserved for difficult cases with multiple varicocele recurrences and is usually coupled with sclerotherapy of the incompetent veins. Ultrasound and thermography studies have comparable sensitivity to venography and are noninvasive, so they are more commonly used to confirm diagnosis of varicocele.We believe that scrotal ultrasonography is not needed if the examination is conducted by a trained fertility physician. However, in circumstances where clinical examination is not conclusive such as obese patients or tight scrotal sac, ultrasonography is needed to confirm the diagnosis.

Conclusion

Varicocele is a common condition that affects both normal and infertile males. It can present with chronic testicular pain, infertility, scrotal swelling, and less commonly hypogonadism. Proper history taking and clinical examination by a trained physician is a pillar for accurate diagnosis and hence legitimate management.

Review Criteria

An extensive search for the literature discussing clinical diagnosis of varicocele was done using scientific search engines, including Pubmed, Medline, ScienceDirect and Google Scholar. Search criteria included the following key words; "varicocele," "scrotal examination," "Infertility," "testicular pain," "scrotal swelling," "hypogonadism," "pathogenesis of varicocele," "grading of varicocele," "guidelines." We included data from published papers or book chapters only. Conference abstracts or communications were not included.

Multiple Choice Questions and Answers

1. Varicocele is usually:
   (a) Painful
   (b) Associated with scrotal swelling
   (c) **Asymptomatic**
   (d) Associated with hypogonadism
2. Varicocele is more common in:
   (a) Patients with hypogonadism
   (b) **Patients with secondary infertility**
   (c) Patients with primary infertility
   (d) Young adults
3. Clinical diagnosis of varicocele should include:
   (a) Medical and surgical history taking
   (b) Physical examination
   (c) Confirmation by radiological investigations in selected cases
   (d) **All of the above**
4. Varicocele pain is characterized by:
   (a) Associated with testicular tenderness
   (b) **Posture dependent**
   (c) Acute in nature
   (d) The most frequent presentation of varicocele
5. Typically, physical examination for varicocele should include:
   (a) Examining the patient in both erect and supine positions
   (b) Examining the spermatic cord with and without Valsalva maneuver
   (c) General examination of the abdomen, pelvis, and perineum
   (d) **All of the above**

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11 Imaging and Other Diagnostic Modalities in Varicocele Diagnosis

Muhannad M. Alsyouf, Phillip K. Stokes, and Edmund Y. Ko

Abbreviations

CT	Computed tomography
MRI	Magnetic resonance imaging
DWI	Diffusion-weighted imaging
ADC	Apparent diffusion coefficient
US	Ultrasonography

Key Points

- Ultrasonography is the imaging modality of choice for the evaluation of the scrotum as it is readily available, cheap, and does not expose patients to radiation.
- The characteristic appearance of a varicocele on an ultrasound is described as "multiple, anechoic, serpiginous, tubular structures," near the superior and lateral aspects of the testis.
- Computed tomography and magnetic resonance imaging are excellent modalities at evaluating retroperitoneal pathology.
- Venography can be used during surgery to guide intraoperative decision-making.
- Scintigraphy is now largely of historical and research interest due to its impracticality.

Introduction

Varicocele is defined as the dilation of the venous pampiniform plexus draining the testicle. Reflux of blood from the internal spermatic vein (ISV) results in dilation of the pampiniform plexus and is thought to be the primary pathologic process for varicocele formation [1]. Reported prevalence of varicocele is estimated at 15% in healthy adult men, and as high as 45% in men undergoing evaluation for infertility [2, 3]. Although the majority of men with varicoceles do not develop infertility, it remains the most common reversible factor in male infertility.

Varicoceles are mainly diagnosed on clinical examination. Various imaging modalities are used by physicians to improve detection rates and provide further anatomic detail of varicoceles.

M. M. Alsyouf (✉) · P. K. Stokes · E. Y. Ko
Department of Urology, Loma Linda University, Loma Linda, CA, USA
e-mail: malsyouf@llu.edu

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However, the benefit of obtaining imaging studies in a subset of varicoceles that are only found on imaging and not appreciated on physical examination has been called into question. Leading authorities including the American Urological Association recommend against the use of routine imaging studies for the detection or screening of subclinical varicoceles in patients without a palpable abnormality [4]. In this chapter, we provide a detailed review of the available imaging modalities and their current role in the evaluation of varicoceles. The imaging modalities discussed in this chapter include ultrasonography, computed tomography, magnetic resonance imaging, venography, thermography and scintigraphy (Table 11.1).

Table 11.1 Summary of available imaging modalities in the evaluation of varicoceles

Imaging modality	Benefit	Disadvantage
Ultrasound	Readily available Relatively inexpensive	Operator dependent No standard diagnostic criteria Significant intra- and interobserver variability
Computed tomography	Excellent at evaluating retroperitoneal pathology	Retroperitoneal pathology as cause of varicocele is rare Expensive Radiation exposure
Magnetic resonance imaging	Excellent at evaluating retroperitoneal pathology No radiation exposure	Retroperitoneal pathology as cause of varicocele is rare Expensive Evidence limited to animal studies in evaluating varicocele
Venography	Adjunct during percutaneous procedures	Invasive
Thermography and scintigraphy	Noninvasive Can be used as adjunct with ultrasound to improve accuracy	No standard diagnostic criteria

Imaging Modalities

Ultrasonography

Ultrasound is the primary imaging modality for the evaluation of diseases of the scrotum [5]. It is readily available, noninvasive, and, since the advent of color Doppler, can provide detailed information regarding vascular flow within the testes and associated structures. Most scrotal pathologies can be identified on physical examination. However, in cases where examination is equivocal, scrotal ultrasonography may be used as an adjunct. This scenario may occur in patients with a tight scrotum or high-riding testes, previous scrotal surgery, or a body habitus that renders physical examination of the scrotum difficult [9]. Figure 11.1 depicts the typical appearance of a varicocele on ultrasound. The characteristic appearance of a varicocele on an ultrasound is described as "multiple, anechoic, serpiginous, tubular structures," near the superior and lateral aspects of the testis [6]. Numerous classification systems exist that allow grading of varicoceles based on ultrasound findings. The most widely accepted systems are the Sarteschi and Chiou classifications and are listed in Tables 11.2 and 11.3, respectively [7, 8].

Ultrasonography is also an excellent tool for investigating any testicular pathology that may concomitantly exist with varicoceles, that is, testicular masses. Additionally, ultrasound can also be used to accurately estimate testicular size and has been shown to be superior to the Prader orchidometer [10]. Objective evidence of discrepancy in testis size in adolescent patients with ipsilateral varicoceles is an indication for varicocele repair as this finding is a sign of varicocele-related testicular injury [11].

There are limitations to ultrasound despite the appeal of utilizing this modality to aid in the diagnosis of varicoceles. To date, there is no consensus on how to assess and standardize the evaluation of varicoceles using ultrasound [12]. Difficulty in standardization is due to its operator-dependent nature. Patient positioning, axis of imaging, employing Valsalva maneuvers, and the methodology of quantifying blood flow are all factors that contribute to significant inter- and

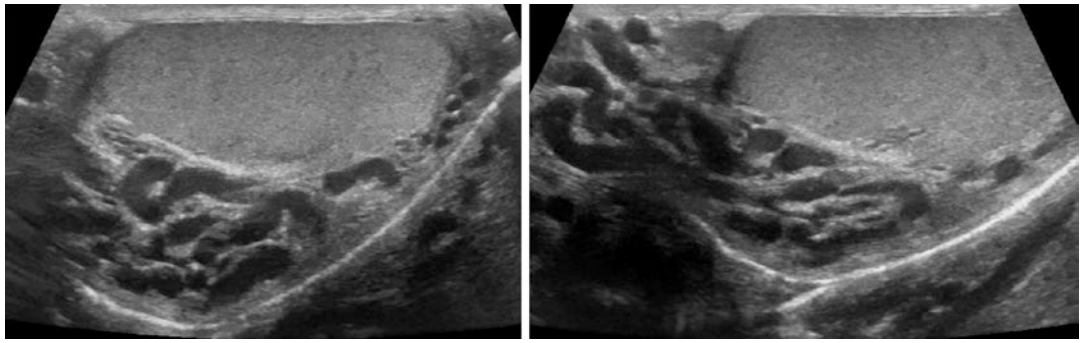

Fig. 11.1 Ultrasound of left testicle demonstrating classic appearance of dilated pampiniform veins consistent with diagnosis of varicocele

Table 11.2 Sarteschi classification for Color Doppler Ultrasound Diagnosis of Varicocele

Grade	Description
1	Venous reflux at the emergence of the scrotal vein only during the Valsalva maneuver; hypertrophy of the venous wall without stasis
2	Supratesticular reflux only during the Valsalva maneuver; venous stasis without varicosities
3	Peritesticular reflux during the Valsalva maneuver; overt varicocele with early-stage varices of the cremasteric vein
4	Spontaneous basal reflux that increases during the Valsalva maneuver, possible testicular hypotrophy, overt varicocele, varicosities in the pampiniform plexus
5	Spontaneous basal reflux that does not increase during the Valsalva maneuver, testicular hypotrophy, overt varicocele, varicosities in the pampiniform plexus

Table 11.3 Chiou et al. scoring system for color Doppler ultrasound diagnosis of varicocele

	Score
Maximum vein diameter (mm)	
<2.5	0
2.5–2.9	1
3.0–3.9	2
≥.0	3
Plexus/sum of diameter veins	
No plexus identified	0
Plexus (+) with sum diameter <3 mm	1
Plexus (+) with sum diameter 3–5.9 mm	2
Plexus (+) with sum diameter ≥6 mm	3
Change of flow velocity on Valsalva maneuver	
<2 cm/s or duration <1 sec	0
2.0–4.9	1
5–9.9	2
≥10	3
Total score	0–9

A total score of 4 or more is used to determine the presence of varicocele on color Doppler ultrasound

intraobserver variability [12]. Additionally, there are no universally accepted defined venous diameters used to diagnose the presence of varicoceles on ultrasonography [13]. The most widely used size criterion to diagnose varicoceles is the presence of multiple veins measuring >3.0 mm with evidence of flow reversal on color Doppler ultrasound with Valsalva [13–15]. However, some authors have suggested that veins as small as 1 mm may be of clinical significance [16–19]. In a study by Pilatz and colleagues, 217 men were investigated to determine the optimal ultrasonographic criteria to detect palpable varicoceles. They found that the optimal cut-off value for venous diameter to predict varicoceles on ultrasound were >2.45 mm in the supine position at rest (sensitivity 84%, specificity 81%) and >2.95 mm and during Valsalva (sensitivity 84%, specificity 84%) [20]. In a similar study by Bakirtas and colleagues, 552 patients were assessed by physical examination and scrotal ultrasound and determined that the optimal cut-off value for venous diameter to predict varicoceles was 3.1 mm (sensitivity 58.2%, specificity 84%) [21].

Demonstration of retrograde flow during Valsalva is another ultrasound feature generally accepted among radiologists as a key finding for the diagnosis of varicocele [12]. In a study examining 127 testes, a new scoring system was developed that included color Doppler ultrasound findings of venous dilation and flow reversal demonstrated a 93% sensitivity and an 85% specificity of identifying varicoceles when compared to physical examination [8]. Despite this evi-

dence, the methodology by which reversal of flow is quantified is not standardized and the added clinical benefit for diagnosis has been controversial [12]. Some centers only recognize the presence or absence of retrograde flow, while others have advocated for measurements of flow velocity. In the study by Bakirtas and colleagues described earlier, the authors also demonstrated that retrograde flow volume cut-off value of 14.5 ml/min was more sensitive than venous diameter in predicting palpable varicoceles, but concluded that neither venous diameter nor retrograde flow volume seemed to have any additional benefit to physical examination in clarifying the diagnosis of suspected low-grade varicoceles [21]. The reliability of demonstrating flow reversal on color Doppler ultrasonography has also been called into question. Cvitanic et al. evaluated the prevalence of varicoceles after surgical repair and in a normal control group. They noted that 64% of postvaricocelectomy patients with no evidence of palpable varicocele on exam did in fact show reversal of flow on color Doppler ultrasound [22]. The authors also noted that 42% of the normal healthy, fertile male control group had evidence of flow reversal on color Doppler ultrasound. Similar discrepancies between physical exam and ultrasound findings were demonstrated by Meacham et al. The group evaluated 34 asymptomatic young men with normal semen parameters and found that only 15% of their cohort had varicoceles detectable by physical examination, whereas 35% were found to have flow reversal on color Doppler ultrasonography [23]. Kocakoc et al. performed color Doppler ultrasonography in 56 healthy men with normal physical exam and semen parameters. They found that more than 50% of these normal males had Doppler evidence of reflux during Valsalva maneuvers [24].

Employing ultrasound in the evaluation of scrotal disease has led to increased detection of subclinical varicoceles that are otherwise undetectable on physical exam. Multiple studies have investigated the benefit of treating subclinical varicoceles in the infertile male. A randomized study by Grasso and colleagues sought to evaluate the benefit of ipsilateral spermatic vein ligation in infertile males diagnosed with low-grade varicocele on US (subclinical) and abnormal semen analysis. Sixty-eight men were randomized into either ipsilateral spermatic vein ligation versus observation. There was no significant improvement in semen quality or paternity rates between the two groups 1 year after surgery [25]. Another randomized prospective controlled study by Yamamoto and colleagues randomly assigned 85 infertile males with subclinical varicoceles to either observation or internal spermatic vein ligation. The group that underwent internal spermatic vein ligation showed higher levels of sperm density and total motile sperm at 1 year; however, there was no significant improvement in seminal volume, sperm motility, abnormal sperm morphology, or pregnancy rates (10% vs. 6.7%; $p = 0.76$) [26]. More recently, Unal et al. compared the effect of varicocelectomy with that of clomiphene citrate on semen improvement and pregnancy rates in patients with subclinical varicocele. There was no difference in pregnancy rates between the surgical group (pregnancy rate 12.5%) compared to the group treated with clomiphene citrate (pregnancy rate 6.7%) ($p = 0.59$) [27].

In summary, although a readily available modality for the evaluation of scrotal pathology, the role of ultrasonography in identifying varicoceles that are otherwise nonpalpable on exam is limited due to the preponderance of evidence indicating no fertility benefit in treatment of subclinical varicoceles.

Computed Tomography

Clinically palpable varicoceles occur on the left side in 85–90% of patients, while isolated right varicoceles are less common [28]. This discrepancy is attributed to the variation in the insertion of the left and right spermatic veins. The left spermatic vein inserts at a right angle into the left renal vein, which is hypothesized to result in higher hydrostatic pressures when compared to the tangential insertion of the right spermatic vein into the inferior vena cava. As a result, right-sided varicoceles and varicoceles that develop acutely

have classically been evaluated with imaging to rule out intra-abdominal pathology [29].

Computed tomography (CT) has gained popularity among physicians since its introduction in the 1970s and has become a mainstay in the evaluation of abdominal pathology. Using submillimeter slice thickness and multiplanar image reconstructions, small vessels including the gonadal veins can be better evaluated than previously possible [30]. There is little published data on multiplanar three-dimensional CT venography as a diagnostic modality for evaluating varicoceles [30]. Although multiplanar CT has been shown to accurately diagnose varicoceles, the increased radiation exposure and the wide-spread availability of ultrasound have made this imaging study less desirable in the evaluation of scrotal pathology.

The role of CT in the evaluation of varicoceles has thus been limited to investigating retroperitoneal pathology and is the study of choice when an abnormality is suspected. Causes of varicocele formation that could be identified on CT imaging include renal tumors, retroperitoneal tumors causing compression, caval thrombi, and retroaortic renal veins [31–34]. The classic teaching has also recommended retroperitoneal imaging in cases of acute onset varicoceles due to concern for retroperitoneal tumors [35]. However, there is a lack of evidence to support this practice, with only case reports and small case series drawing an association between right-sided or acute-onset varicoceles with the presence of renal or other retroperitoneal tumors [29].

Magnetic Resonance Imaging

Diffusion-weighted imaging (DWI) has enabled magnetic resonance imaging (MRI) to detect early changes within tissue parenchyma before they can be visualized grossly on traditional imaging modalities [36]. Multiple advances have enabled this modality to find application for non-urologic pathologies. In contradistinction to traditional magnetic resonance studies, diffusion-weighted imaging studies are fast and do not require intravenous contrast infusion [36]. Quantitative analysis of DWI can be obtained by calculating the apparent diffusion coefficient (ADC). For example, tissue that is highly cellular and thus possesses a greater amount of molecular barriers to water diffusion will show a lower apparent diffusion variable. Conversely, areas that have a paucity of cells will yield a higher ADC [36]. In the evaluation of scrotal pathology, the use of MRI has been investigated and shows promise. Utilizing ADC measurements, multiple animal studies have shown that MRI can reliably differentiate ischemic from nonischemic tissue [37, 38].

Numerous studies have also examined the appreciable changes in the testicular parenchyma as measured using diffusion-weighted MRI in patients with varicocele. In a study by Karakas et al., 25 patients with varicocele were matched with 25 healthy controls [39]. The patients were examined by a urologist and confirmed to have a varicocele by palpation as well as by ultrasound. Subjects were then examined using a 1.5-tesla MRI machine and the imaging data were analyzed to yield ADC measurements. In patients who were diagnosed with varicocele, the mean ADC values were significantly lower in those with larger venous diameters at rest and during Valsalva. The ADC values of the ipsilateral testicular parenchyma were lower than those of controls. Furthermore, the ADC values of the parenchyma in the contralateral testis in patients with varicocele were also lower than healthy controls [40]. Given these findings, it would be reasonable to apply diffusion-weighted MRI to the detection of testicular fibrosis and resultant sequelae of varicoceles in the support of intervention.

Yıldırım et al. also showed correlation between ADC and varicocele [41]. The authors examined the use of the ZOOMit (Siemens Healthcare) diffusion-weighted magnetic resonance, because standard diffusion-weighted magnetic resonance modalities use a single radiofrequency pulse sequence and may lead to image distortion. ZOOMit uses a second concurrent parallel pulse to allow for more anatomic detail. In this series, 45 patients with varicocele were matched with 32 healthy controls. ZOOMit as

well as diffusion-weighted images were obtained. ADCs from both modalities were lower in both the testis with varicocele and the contralateral testis. The ZOOMit ADC values were significantly lower in testes with varicocele although significance was not achieved with conventional ADC calculation. The authors also discovered negative correlation between ZOOMit ADC values and venous diameter at rest and with Valsalva in the testis affected by varicocele.

Furthermore, it has been shown that MRI has potential applications in quantifying the effect of varicocele on fertility. A small pilot study Çekiç et al. showed that decreased ADC values in patients with varicoceles correlated with semen parameters [42]. Thirty-one patients with varicoceles were matched with 20 healthy controls. In those with varicocele, there was a negative correlation between the mean ADC value and the pampiniform plexus vein diameter. In addition, there was noted to be a positive correlation between the mean ADC values and sperm count as well as sperm morphology.

Although the above-described MRI modalities are able to elucidate the effects of varicocele on the testicular parenchyma without the use of intravenous paramagnetic contrast, dynamic contrast-enhanced MRI evaluates the distribution of contrast within a tissue of interest. Normal testicular tissue enhances in a linear and homogenous fashion, which some have speculated is due to the immunologically privileged nature of the testicle. Using gadolinium-based contrast, this modality has been described for use in distinguishing benign from malignant testicular lesions [43]. Alterations of testicular perfusion due to disruptions in this barrier in testis with varicoceles may be detected by using this modality [44].

While not commonly used in clinical practice, magnetic resonance venography (MRV) has a place in certain scenarios. An example is the young patient in whom reduction of radiation is a primary goal and those who may be allergic to contrast agents [45]. Varma and colleagues described the use of MRV in an adolescent with recurrent varicoceles who had undergone multiple interventions. The authors felt that MRV would obviate the need for radiation and invasive diagnostic procedures without any promise of benefit. They performed three-dimensional phase contrast MRV to delineate a single patent ISV, which allowed for targeted coil embolization of this vessel as well as pelvic collaterals [46].

Venography

Today, percutaneous angiographic imaging techniques related to varicoceles are not typically used as a diagnostic modality in the absence of an interventional procedure. Their use for diagnosis alone is largely of historical and research interest. Fluoroscopic evaluation of the venous drainage of the testicle was first performed and described by Ahlberg et al. in 1966 [47]. He demonstrated retrograde blood flow within the gonadal veins by percutaneous cannulation of the femoral vein. Although initially proposed by Comhaire and Kunnen in 1976, Lima et al. described a therapeutic procedure of cannulating the left spermatic vein via percutaneous access from the left femoral vein as a technique for treatment of varicocele in 1978 [48, 49]. Since then many advances have been made. This initial approach was limited by reduced access to the right spermatic vein. Later progress was made, which allowed easier access to the right via a transjugular approach [50].

In today's practice, venography is a useful adjunct, which may guide intraoperative decision-making. Figure 11.2 depicts venography of the left spermatic vein with subsequent successful percutaneous coil and sclerosant embolization for the treatment of a persistent symptomatic varicocele that failed subinguinal spermatic vein ligation. Understanding of the venous anatomy and variations thereof is paramount to interpreting and using venography. The venous drainage of the testicle is accomplished by the pampiniform plexus, a network of small veins that receives blood from the ipsilateral testis. The multitude of veins consolidates and drains into the gonadal vein, which drains into the renal vein on the left and the inferior vena cava on the right. There are well-recognized

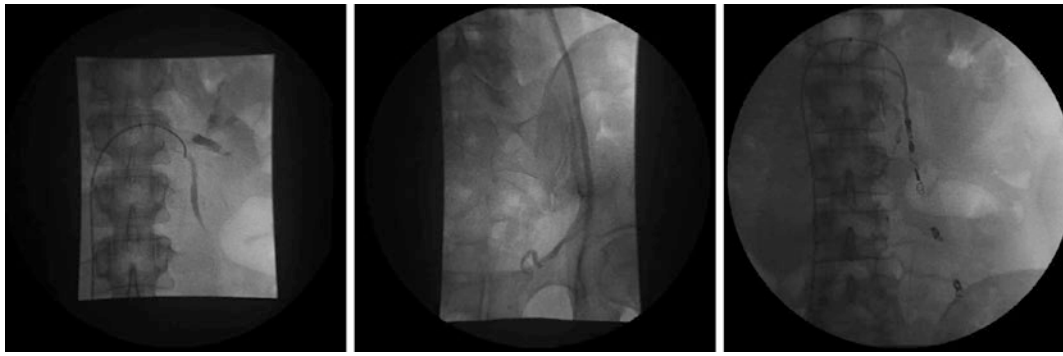

Fig. 11.2 Venography of the left spermatic vein with subsequent successful percutaneous coil and sclerosant embolization for the treatment of a persistent symptomatic left varicocele

variations on the right side including drainage of the gonadal vein into the right renal vein, and several veins draining into the vena cava and right renal vein. On the left, multiple veins may be found draining into the left renal vein. Furthermore, testicular venous drainage may also include the external pudendal vein, which drains into the great saphenous vein [51].

Venography has also been described for situations where varicoceles recur following definitive surgical therapy. Morag et al. described using venograms in 40 patients with recurrent varicocele or abnormal semen analysis following high ligation of the left spermatic vein [52]. Recurrence after percutaneous ablation or surgical ligation has been demonstrated to be secondary to collateral veins, which bypassed the occlusion. In a series by Kaufman et al., the gonadal vein was noted to reconstitute in the pelvis of 5 out of 8 patients following balloon occlusion and in all patients following ligation [53]. Furthermore, salvage venography has been described in the setting of failed surgical ligation or percutaneous ablation. Murray et al. published a series of 44 recurrent varicoceles in 37 patients treated with both surgical and percutaneous interventions [54]. They demonstrated three types of recurrence patterns, namely, parallel, renal vain and trans-scrotal collateral circulations. Recurrence following ligation was secondary to either retroperitoneal (27%) or inguinal parallel collaterals (58%). Sze et al. investigated persistent varicoceles in a series of 17 patients evaluated by retrograde venography. All patients had persistent or recurrent varicoceles, which were studied 4 months to 18 years following open surgical repair. Most of the patients had duplications draining into a single left gonadal vein, which were most commonly found in the pelvis or inguinal canal. All patients underwent embolization with N-butyl cyanoacrylate (NBCA) or coils or a combination. At 6 months, the procedure was successful at reducing symptoms in all patients [55].

Thermography and Scintigraphy

Although the understanding of pathophysiology of varicoceles with respect to infertility has evolved, initially, it was widely believed that heat was the inciting cause of abnormal semen function [56]. This understanding prompted the use of scrotal thermography to identify areas of hyperthermia as a screening tool for subclinical varicoceles. Thermography measures the temperature at the surface of scrotal skin and utilizes a film with heat-sensitive liquid crystals (Fig. 11.3) [49]. As has been demonstrated, renal venous blood, which refluxes into the internal spermatic vein, is warmer than blood exiting the pampiniform plexus [57].

In 1970, Kormano et al. described the technique of scrotal thermography comparing men with varicoceles with healthy controls [58]. In this series, subjects acclimatized for 10 minutes at 22–23 °C following which the thermographic camera was placed at a distance of approximately

Fig. 11.3 Clinically occult varicocele, left side of the scrotum. The heat from the side of the varicocele exceeds that from the normal side by 4.5 0 C (80 F). The patient manifested only a decreased sperm count. (Reprinted from Gold et al. [71]. With permission from Radiological Society of North America)

40 cm from the scrotum. Images were obtained standing at rest, during, and shortly after a Valsalva maneuver. In 1976, scrotal thermography was compared to venography for the diagnosis of subclinical varicocele by Comhaire et al. [49]. The authors in this series found that among 39 patients with a varicocele present on physical exam, 37 had abnormal thermograms. Of the 36 men with possible subclinical varicocele on examination, 19 had abnormal thermograms and 16 had reflux on venography.

In addition to traditional infrared thermography, which requires complex and potentially costly instruments, contact thermography has been investigated using a disposable strip; however, it yielded contradictory results and is no longer offered commercially [59]. Contact thermography was also compared with ultrasound to the gold standard of retrograde venography by the WHO Task Force on Diagnosis and Treatment of Infertility. In this multicenter series of 141 men with infertility, ultrasound combined with contact thermography had the highest diagnostic accuracy with a 1% false-negative result and 44% false-positive results [60].

Hamm et al. also investigated the combination of thermography with ultrasound prior to percutaneous venography of the internal spermatic vein [16]. They found that the accuracy of infrared thermographic measurements in identifying varicocele was 98.4% with ultrasound being 92.7% accurate. Others have noted sensitivities of 84–94% and specificity of 81–100% [59].

It is also important to note that caution should be exercised when interpreting results of scrotal thermography in isolation of physical exam or other diagnostic modalities. Although normothermia on thermogram is rarely found in the setting of varicoceles, hyperthermia may also be the result of inflammatory conditions such as epididymitis [59].

Scintigraphy remains a diagnostic modality worth mentioning although now largely of historical and research interest due to the time required and impracticality compared to other modalities. Various methods exist for performing scrotal scintigraphy. Vanlangenhove et al. described a proposed method [59]. The patient's red blood cells (RBCs) are labeled by intravenous administration of pyrophosphate 20 mCi prior to administration of 99 m Tc pertechnate. The patient is then examined standing with the penis secured to the abdomen in the midline. The scrotum is located in the lower third of area of interest, while a gamma camera evaluates the accumulation of radioactive tracer labeled red blood cells.

From an objective standpoint, scintigraphy has distinct diagnostic advantages with respect to sensitivity and specificity. Scintigraphy is able to provide dynamic imaging and quantitative information on the amount of reflux in the internal

spermatic vein. Furthermore, it may be used to differentiate between the etiologies: reflux versus extrinsic compression of pelvic veins [59].

Chen et al. evaluated radionucleotide scrotal imaging compared to physical exam and found that scintigraphy had rates of sensitivity and specificity that surpassed physical exam:96.5% and 97.1% versus 71.7% and 69.1%, respectively [61]. Scintigraphy has also been shown to be equivalent to thermography and ultrasound when compared to the gold standard of venography. A series of 163 patients with varicoceles were investigated by Geatti et al. who found the correlation between a positive result from venography was 98% for scintigraphy, 100% for thermography, and 98% for ultrasound [62].

Future Perspectives

Molecular and genetic factors have been investigated to attempt to identify patients with the diagnosis of varicoceles that will ultimately develop infertility and require treatment. It has been postulated that men with varicocele-associated infertility may have pre-existing genetic lesions and molecular mechanism defects that render them more susceptible to varicocele-mediated testicular injury affecting spermatogenesis [63]. One of the molecular mechanisms that have been investigated are heat-shock proteins, which are molecular chaperons with protective action on cellular autoregulation that are produced by cells in response to exposure to heat stress [64]. In a study by Lima et al., evaluation of ejaculated spermatozoa demonstrated that gene expression of heat-shock protein A2 was downregulated in sperm from adolescents with varicocele and oligozoospermia compared to adolescents with varicocele and normal

sperm concentration [65]. Similarly, Ferlin and colleagues analyzed the expression of heat-shock proteins in the sperm of men with normozoospermia and oligozoospermia with or without varicocele. The study reported that expression of HSFY gene, a heat-shock protein, was upregulated only in men with varicocele and normal semen parameters, suggesting that it may represent a molecular marker for the response to the effect of varicocele on spermatogenesis [66]. Other heat-shock proteins have also been hypothesized to play a role in the pathophysiology of varicoceles [67]. Cervellione and colleagues measured peripheral venous levels of basal thiobarbituric acid reactive substances (TBARS) and plasma peroxidation susceptibility (which are useful to assess lipid peroxidation) in adolescent patients with left-sided varicocele and ipsilateral testicular hypoplasia. The study demonstrated a reduction in basal TBARS and an increase in the plasma peroxidation susceptibility lag time after varicocelectomy, suggesting that varicocelectomy reduced oxidative stress in these patients [68]. In the future, peripheral blood measurement of oxidative stress markers may potentially provide helpful information to guide decisions on treatment for adolescents with varicoceles.

Although additional investigation of molecular and genetic factors related to varicocele-related testicular injury is needed, studies to date have showed promising evidence and suggest that these markers may play a role in the future in the decision to treat adolescent varicocele patients [64].

Conclusion

Clinical history and physical exam remains the mainstay of the diagnosis of varicocele. Ultrasound remains the most widely used modality in evaluating scrotal pathology, and this is likely because it is readily available, relatively inexpensive, and a noninvasive modality. Drawbacks include no universally accepted standardized ultrasonographic parameters available to identify meaningful varicoceles, and the significant inter- and intraobserver variability in performing these studies. Although CT is an excellent modality in identifying retroperitoneal pathology causing secondary varicoceles, this association is uncommon, usually presents late in the disease process, and studies demonstrating this association have been limited to case reports and small case series. At this time, MRI is mainly an investigational tool, which does have broad

potential for identifying which men may benefit from intervention depending on the degree of testicular fibrosis. Scintigraphy and thermography have largely been relegated to the historical context. Table 11.1 summarizes the advantages and disadvantages of the imaging modalities available for the evaluation of varicoceles.

A defined role of imaging in the evaluation of varicoceles remains to be determined. The American Urological Association Best Practice Statement on Male Infertility supports ultrasound imaging only in cases where physical examination is difficult or inconclusive [69]. While European Association of Urology Guidelines on Male Infertility advocate for the use of ultrasound for confirmation of physical exam and the use of venography in centers where treatment is carried out using percutaneous methodologies [70].

To date robust data to support routine use of any imaging modality in the initial evaluation of patients with varicoceles is lacking.

Review Criteria

A comprehensive search of studies examining literature examining imaging studies and varicoceles was performed using search engines such as ScienceDirect, OVID, Google Scholar, PubMed, and MEDLINE. The overall strategy for study identification and data extraction was based on the following key words: "varicocele," "infertility," "ultrasound," "magnetic resonance imaging," "computed tomography," "venography," "thermography," and "scintigraphy." Data that were solely published in conference or meeting proceedings in abstract form or websites were not included. Websites and book-chapter citations provide conceptual content only.

Multiple Choice Questions and Answers

1. Subclinical varicoceles are defined as
   (a) **Varicoceles only appreciable on physical exam**
   (b) Varicoceles only appreciable on imaging
   (c) Varicoceles that cause only pain but do not impact fertility
   (d) Varicoceles that only impact fertility but do not cause pain

   Answer: (a). Subclinical varicoceles are not appreciable on exam but are diagnosed on imaging. Usually, imaging of the scrotum is performed when patients present with a normal physical exam but complains of pain or inability to conceive, but subclinical varicoceles can also be identified in asymptomatic patients.
2. According to American Urological Association Best Practice Policy for Male Infertility, all of the following are indications for scrotal ultrasonography in the evaluation of an infertile male except
   (a) Inconclusive physical exam
   (b) Suspicion of testis mass associated with varicocele
   (c) **As part of routine evaluation of any infertile male**
   (d) None of the above

   Answer: (c). Ultrasound evaluation of the scrotum is not indicated in the routine evaluation of an infertile male as multiple studies have shown that treatment of varicoceles only seen on ultrasound does not improve fertility. Indications for obtaining ultrasound imaging include inconclusive exam including patients with testes that are in the upper scrotum, previous scrotal surgery, or abnormal body habitus. Additionally, any suspicion of an associated testis neoplasm warrants further evaluation with ultrasound.
3. A 32-year-old infertile man has a semen volume of 1.5 ml and decreased sperm motility. Physical examination demonstrates a grade 3 left varicocele with ipsilateral decrease in testis size on examination. His partner's evaluation is normal. The next step is
   (a) Transrectal ultrasonography
   (b) Venography
   (c) Scrotal ultrasonography
   (d) **Left varicocelectomy**

   Answer: (d). This infertile patient has abnormal semen parameters and a palpable varicocele with discrepancy in testis size on the ipsilateral size, which is an indication of vari-

cocele repair as studies have shown improvement in semen parameters. This benefit has not been shown in patients with a subclinical varicocele. There is no indication for additional imaging as the varicocele and size discrepancy are appreciable on exam.

4. Which of the following imaging modalities can be used as an adjunct during fluoroscopic percutaneous embolization of varicoceles?
   (a) **Venography**
   (b) Thermography
   (c) Scintigraphy
   (d) Ultrasound

   Answer: (a). Venography is seldom performed for the sole purpose of diagnosing varicoceles given the invasive nature of this procedure. Venography, however, is employed during percutaneous embolization of varicoceles as it can provide anatomical details during the procedure that may guide intraoperative decision-making.
5. Advantages of Computed Tomography over ultrasound imaging include all of the following except
   (a) Detailed evaluation of retroperitoneal tumors
   (b) Better at identifying variations in renal vein anatomy (i.e., retroaortic left renal vein)
   (c) **Less radiation exposure**
   (d) A and C

   Answer: (c). The main risk of CT imaging is the radiation exposure to patients. Otherwise, computed tomography is an excellent study at evaluation of retroperitoneal tumors as well as identifying variations in renal vein anatomy such as a retroaortic renal vein.

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12 Conventional Semen Analysis and Specialized Sperm Function Tests in Patients with Varicocele

Ahmad Majzoub, Chak-Lam Cho, Ashok Agarwal, and Sandro C. Esteves

Key points

- Quality assurance and standardization protocols are fundamental parts of the andrology laboratory: if these are adequately applied to conventional semen analysis, sound correlations with natural conception and assisted reproductive outcomes may be obtained.
- While fertile and infertile men with varicocele may have similar semen parameter results to those without varicocele, current evidence demonstrates a significant and well-established link between varicocele and male infertility.
- Varicocelectomy should be offered as the first-line treatment for clinical varicocele in subfertile men.
- Among the various sperm function tests, sperm DNA fragmentation tests and measures of oxidative stress have been evidently associated with spermatogenic dysfunction and male infertility.
- OS markers are significantly higher in varicocele patients and significant reduction in seminal OS has been reported after varicocelectomy.
- DNA damage in sperm cells has been linked to poor semen quality, impaired preimplantation development, and increased abortion.

Introduction

Varicocele is one of the most common conditions causing male infertility [1]. It is highly prevalent in infertile patients, which prompts the need for optimal diagnostic tests to direct management and to monitor intervention outcomes. Conventional semen analysis is no longer sufficient alone in the laboratory assessment of men with varicocele. It was shown that sperm concentration and motility might increase with time in oligozoospermic and asthenozoospermic men even in the absence of treatment [2, 3]. This improvement in sperm analysis parameters is explained by the "regression toward the mean" phenomenon, which is a mathematical event that

A. Majzoub (✉)
Department of Urology, Hamad Medical Corporation, Weill Cornell Medicine Qatar, Doha, Qatar

C.-L. Cho
S.H. Ho Urology Centre, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

A. Agarwal
American Center for Reproductive Medicine, Department of Urology, Cleveland Clinic, Cleveland, OH, USA

S. C. Esteves
ANDROFERT, Andrology & Human Reproduction Clinic, Campinas, SP, Brazil

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occurs with no relation to biology [4, 5]. In addition to its intrinsic variability, technical and laboratory variations in semen analysis results are yet other factors that may jeopardize our ability to rely solely on semen analysis for varicocele repair decisions. Moreover, routine semen analysis cannot ascertain the functional ability of a given semen sample to fertilize the human ovum in vitro or in vivo, though it quantitates sperm concentration, motility, and morphologic features [6]. Hence, clinicians started relying on ancillary tests of sperm function to aid in decision making. In recent years, specialized tests of sperm function including measures of DNA integrity and oxidative stress (OS) became powerful tools. This mainly stemmed from the remarkable evidence retrieved from animal and human studies showing significant association among sperm DNA fragmentation (SDF), OS, and male infertility. SDF is believed to alter fertilization, embryogenesis, and pregnancy rate. OS can impair sperm parameters and aggravate DNA damage. Despite the profound development in this field, further research is still required to standardize the protocols, validate test results in large clinical trials, and evaluate the cost-effectiveness of such modalities. Proteomics, metabolomics, and genomics are new promising areas to revolutionize the understanding of reproductive physiology, including varicocele.

The main objective of this chapter is to clarify and discuss the relevance of conventional semen analysis and specialized sperm function tests in the context of varicocele and its repair.

Conventional Semen Analysis

Semen analysis is the most globally used biomarker to estimate male fertility potential, to monitor a treatment affecting testicular or accessory gland function, following vasectomy or in a research context [7]. Accordingly, health care providers depend on it to determine if further investigation of the male partner is required. Routine semen analysis includes the evaluation of different parameters such as physical characteristics of semen (liquefaction, viscosity, pH, and volume), sperm concentration, sperm motility and progression, sperm morphology, and leukocyte quantification.

The Semen Analysis (WHO Criterion)

The semen analysis is the cornerstone of the assessment of the male partner for infertility that evaluates not only spermatozoa, but also seminal plasma and non-sperm cells. Previous World Health Organization (WHO) manuals issued in 1987 [8], 1992 [9], and 1999 [10] for the examination of human semen introduced reference or normal values, which caused confusion among clinicians and resulted in over- or underdiagnosis. The main reasons behind such uncertain values were that the data were derived from imprecisely defined normal reference populations, and that the laboratories involved in semen analysis used incomparable analytical methods [11].

To establish evidence-based reference values, the WHO 2010 manual obtained the values from 1953 fertile men in eight countries who became fathers with a time to pregnancy of less than 12 months [12]. In the updated fifth edition of the manual, new methods to measure ejaculate volume by weight and assess sperm morphology, sperm count, sperm motility, and quality control routines were included [12]. The 95% interval for sperm volume, count, motility, vitality, and morphology were generated, and the fifth centile was proposed as the lower cutoff limits (Table 12.1) [12].

Quality assurance is an integral part of any laboratory to ensure reliable clinical results [13]. After the introduction of quality assurance into the andrology laboratories, the results of semen analysis correlated well with natural conception and assisted reproductive technologies [13]. This can be explained by the implementation of international standardization for the entire sperm morphology evaluation procedure, robust training, and the installation of international external quality control (EQC) schemes [14, 15]. In some settings, especially in developing countries, clinicians still rely on the results of the semen analysis to reach a

Table 12.1 Cutoff reference values for semen characteristics as published in consecutive WHO manuals

Semen characteristics	WHO 1980	WHO 1987	WHO 1992	WHO 1999	WHO 2010[a]
Volume (mL)	ND	≥2	≥2	≥2	1.5
Sperm count (10^6/mL)	20–200	≥20	≥20	≥20	15
Total sperm count (10^6)	ND	≥40	≥40	≥40	39
Total motility (% motile)	≥60	≥50	≥50	≥50	40
Progressive motility[2]	≥2[3]	≥25%	≥25% (grade a)	≥25% (grade a)	32% (a + b)
Vitality (% alive)	ND	≥50	≥75	≥75	58
Morphology (% normal forms)	80.5	≥50	≥30[4]	(14)[5]	4[6]
Leukocyte count (10^6/mL)	<4.7	<1.0	<1.0	<1.0	<1.0

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ND not defined
[a]Lower reference limits generated from the lower fifth centile value
[b]Grade a = rapid progressive motility (> 25 μm/s); grade b = slow/sluggish progressive motility (5–25 μm/s); Normal = 50% motility (grades a + b) or 25% progressive motility (grade a) within 60 min of ejaculation
[c]Forward progression (scale 0–3)
[d]Arbitrary value
[e]Value not defined but strict criterion is suggested
[f]Strict (Tygerberg) criterion

diagnosis [7, 15]. Therefore, quality assurance and standardization protocols are fundamental parts of the andrology laboratory. However, despite improvements in the training of laboratory scientists, the techniques of semen analysis are still poorly implemented at many locations.

Evaluation of Conventional Semen Analysis in the Context of Varicocele

The exact mechanisms behind the effects of varicocele on semen quality remain uncertain. Spermatic venous reflux creates a hostile environment to spermatogenesis, which results in reduced quality of the sperm production and even azoospermia in some cases [16]. Infertility in men with varicocele can be attributed to different possible mediators including, scrotal hyperthermia, oxidative stress, hormonal disturbances, testicular hypo-perfusion, testicular hypoxia, and backflow of toxic metabolites [16, 17]. However, it was shown that fertile and infertile men with varicocele have similar semen parameters to those without varicocele [6, 18]. This means that varicocele affects semen quality and fertility in some but not all patients. The discrepancy can be explained by the assumption that varicocele does not affect sperm quality but simply coexists in some males with idiopathic infertility and abnormal semen parameters. Another explanation for this discrepancy is that the genetic transcriptional response to the state of OS seen in some men with varicocele might result in sperm protection against damage. It was shown that HIF-1 protein is expressed in the mouse testis under normoxic conditions, which has made this transcription factor a target of interest [19]. HIF-1 binds to the hypoxia-response elements in the promoter region of target genes, which leads to increases in those genes' expression [20]. Preliminary evidence suggests that HIF-1 may play a role in normal Leydig cell function [21]. However, investigation of whether this is applicable to OS generally and to the specific conditions of the testis, including varicocele, is required.

Despite previous beliefs, current evidence demonstrates a significant and well-established link between varicocele and male infertility. The adverse effects of varicocele on spermatogenesis have been demonstrated by the progressive reduction in the testicular size ipsilateral to the varicocele [22]. Since Macleod's work that revealed an association among infertility, abnormal semen parameters, and varicocele, many studies have examined varicocele effects on semen analysis [2, 3]. It was shown that varicocele can influence all of semen parameters including sperm concentration, sperm motility, and sperm morphology [23, 24].

In order to evaluate the effect of varicocele on semen parameters, a study was conducted in the early 1990s, by the World Health Organization (WHO), involving 9034 men. Based on the 1989 WHO manual utilized at that time, the study revealed significant decreases in the mean total sperm count per ejaculate, percentage of sperm with motility, and percentage of morphologically normal sperm in men with varicocele compared to controls without varicocele [25]. However, the study did not include a control group of healthy men with proved or unproven fertility or account for the magnitude of changes between patients and controls. To accommodate for these drawbacks and to establish if the negative effects of varicocele on semen parameters are maintained with the 2010 WHO manual, a recent meta-analysis was conducted based on ten studies. The studies included in the analysis reported the actual semen parameters of adult infertile men diagnosed with clinical varicocele and contained a control group of either fertile men or normozoospermic men who were not diagnosed with varicocele. The meta-analysis demonstrated that varicocele was associated with significantly reduced sperm count, motility, and morphology but not semen volume [23].

In another cross-sectional study of 5447 Chinese men with varicocele, varicoceles were shown to be independently associated with semen volume, sperm concentration, proportion of sperms with normal morphology, motility, total sperm count, and forward movement sperm count [26]. This recent evidence signifies varicocele as a detrimental risk factor that negatively affects semen quality and individual sperm parameters.

Association Between Conventional Semen Analysis and Varicocele Grade

Studies assessing the relationship between varicocele grade and disturbance of semen analysis components are still scarce. The available data ensures that varicocele grade is strongly associated with sperm morphology, concentration, and sperm motility and that an inverse association is found between the varicocele grade and the semen analysis component [27].

There is no difference in progressive sperm motility or median sperm concentration in patients with Grade 1 varicocele compared to those with Grade 2 varicocele, but the difference is significant in comparison to Grade 3 varicocele patients, where sperm motility and sperm concentration are lower [27, 28]. In a cross-sectional multicenter study from six European countries, increasing varicocele grade was associated with poor semen quality, even in Grade 1 varicocele [25]. When grouped according to low-, intermediate-, or high-quality semen group, the presence and increasing grade of varicocele resulted in higher proportion of men categorized as having low semen quality [25]. In Grade 3 varicocele, sperm concentration is usually less than half of that in men with no varicocele [25].

Conventional Semen Analysis and Varicocele Repair

Varicocelectomy for the treatment of varicocele-induced infertility indicates a remarkable improvement in fertility profile [29]. However, clinical trials concerning its effectiveness reveal conflicting results and consequently, varicocelectomy has been criticized especially under the light of evidence-based medicine [30, 31].

Earlier studies exploring the effect of varicocelectomy on male fertility reported no significant improvement in semen parameters or pregnancy rates following surgery [32–34]. These studies however were criticized as being suboptimal especially that they have included men with normal semen analyses and with subclinical varicocele. On the contrary, varicocelectomy in randomized clinical trials achieved significant improvements in semen parameters. Sperm concentration increased by 75%, whereas motility and morphology by 5.2% and 8%, respectively [35].

Varicocelectomy leads to significant improvements in sperm count and motility regardless of the chosen surgical technique [6, 36]. When comparing high ligation of the veins to subinguinal microsurgical varicocelectomy, there was a consistent improvement in sperm concentration,

motility, and morphology [36]. The sperm concentration increased significantly by $9.71 \times 106\ ml^{-1}$ and motility increased by 9.92% after microsurgical varicocelectomy [37]. Similarly, the sperm concentration increased by $12.03 \times 106\ ml^{-1}$ and motility increased by 11.72% after high ligation varicocelectomy [37]. The improvement in sperm morphology was 3.16% after both microsurgery and high ligation varicocelectomy [37]. In clinical varicocele patients with subfertility and/or at least one abnormal semen parameter, high ligation, inguinal varicocelectomy, and subinguinal varicocelectomy led to a significant improvement in sperm count and motility, with only minimal differences observed between intervention groups [38]. The higher increment in sperm count and sperm motility was achieved by inguinal approaches, with no clinical significance when compared to other techniques [36]. Nonetheless, recent evidence extracted from meta-analyses has demonstrated lower complication rates characterized by varicocele recurrence and hydrocele formation with microsurgical subinguinal varicocelectomy, suggesting a slight precedence for this surgical approach [39, 40].

Repair of clinical varicoceles in oligozoospermic patients indicates that varicocelectomy is moderately superior to observation regarding pregnancy outcomes, but the effect is not statistically significant [41]. The improvement in the natural pregnancy rates after varicocelectomy correlates negatively with the duration of infertility [41]. Therefore, duration of infertility should be considered in treating a patient with a varicocele as a cause of infertility.

Varicocelectomy improves sperm motility and concentration in all varicocele grades, but mostly among Grade 3 patients and especially after 6 months of surgery rather than 3 months [28]. Patients who have larger varicoceles have greater improvements in semen analysis parameters after the procedure than men who had smaller varicoceles [28]. However, the percentage of pregnancy rates is more in lesser grades of varicocele [28].

Hence, varicocelectomy is associated with significant refinements in sperm concentration, morphology as well as total and progressive motility. There is conclusive evidence that a varicocele repair improves natural pregnancy rates. A meta-analysis suggests that surgical varicocele repair plays a significant role in improving the pregnancy rate when performed in men with clinical varicocele and abnormal semen parameters [42, 43]. Therefore, surgical repair should be offered as the first-line treatment for clinical varicocele in subfertile men.

Specialized Sperm Function Tests

Conventional semen analysis classifies infertility according to the type and degree of spermatogenetic effect, but provides limited information about the sperm function in vitro or in vivo [44]. In addition, there is high inter- and intraobserver variability in processing and analyzing during semen analysis, especially regarding sperm morphology, which is correlated with pregnancy outcomes [45]. More importantly, semen analysis has poor prediction of fertility, since 50% of infertile men have normal semen parameters and patients with abnormal semen analysis can be fertile [41]. This necessitates the need for more comprehensive sperm function tests to identify the sperm dysfunction at the cellular and molecular levels. Therefore, advanced sperm function tests have been developed and introduced into clinical practice. These tests use different methods and techniques to evaluate different stages of fertilization as noted below.

Effects of Varicocele on Sperm Function and Semen Oxidative Stress

Free radicals, known as reactive oxygen species (ROS), are normally formed during the intermediate steps of cellular metabolism. A physiologic level of ROS is essential for regulation of sperm capacitation, and a balanced cellular environment is maintained by the presence of enzymatic and nonenzymatic antioxidant-scavenging systems [46–48]. ROS are unstable and highly reactive molecules that target cell membranes and

increase peroxidation of membrane polyunsaturated fatty acids [49]. These effects are detrimental to the structure of sperm head and midpiece membrane, ultimately leading to suboptimal motility and fertilization [49]. Damage to axonemal proteins and spermatozoal nuclear and mitochondrial DNA are other important sites of action of ROS [49].

Recently, OS has been implicated as a key element in the pathophysiology of varicocele-associated infertility [50]. OS is the result of imbalance between ROS and a protective antioxidant system. Varicocele and OS association was demonstrated by the higher levels of ROS and lipid peroxidation products in infertile men with varicocele compared to infertile men without varicocele [50, 51]. Even fertile men with varicocele are more likely to have elevated OS compared to those without varicocele [51]. Varicoceles are thought to induce the elevation of scrotal temperature, which increases ROS production from mitochondria, plasma membrane, cytoplasm, and peroxisome in the presence of heat stress [52]. Also, infertile men with varicoceles have higher incidence of leukocytospermia, and leukocytes are a major source of ROS in the presence of cytokines and inflammatory cells [53, 54]. Varicoceles are associated with elevated cadmium levels in testicular biopsy samples of infertile patients, as well. Cadmium has negative effects on spermatogenesis, since it reduces zinc concentration and enhances ROS production [55]. In addition, hypoxia and heat stress can trigger various cell types lining the epididymal tubules that are capable of generating ROS to increase their production levels [56–59]. Moreover, ROS are major causes of SDF due to damage to both mitochondrial and sperm nuclear DNA [59]. Fertile and infertile men with varicocele have higher SDF than those without varicocele, meaning that varicocele itself is associated with DNA damage even when fertility has not been compromised. Also, excessive ROS can induce apoptosis in mature spermatozoa, resulting in persistently abnormal spermatozoa [51].

Sperm Function Tests Utilization Among Varicocele Patients

Specialized semen tests are required to elucidate the etiology of subfertility in a subset of patients. These tests are important to determine specific defects of human sperm physiology (Table 12.2). Despite their clinical usefulness, more information is needed to determine if these tests will truly predict fertility potential and if they can explain unexplained infertility.

Antisperm Antibodies

Antisperm antibodies (AsAbs) are typically produced as a result of disruption of the blood–testis barrier, allowing the patient's own immune system to identify the haploid sperm cells [60]. Other possible causes may include inoculation of the host with sperm antigens, failure of immunosuppression, and acute and chronic prostatitis [60]. AsAb affects normal fertilization through interfering with sperm agglutination, cervical mucus penetration, and sperm–oocyte interaction [60, 61]. AsAb may interfere, as well, with sperm differentiation, capacitation, acrosome reaction, zona binding and penetration, or sperm–oocyte membrane interactions [60, 61].

AsAb has low positivity among clinical varicocele patients, as they were only detected in approximately 30% men with clinical varicocele [62]. The production of AsAb in varicocele patients is not clear. In unilateral experimental varicocele, the blood–testis barrier remained intact, but deterioration of testicular function was reported [63].

Despite being present in 3–12% of infertile men, antisperm antibodies (AsAbs) are also present in high levels among fertile men [64]. This complicates the clinical significance of AsAb in the diagnosis of subfertility and its treatment.

Various tests can be carried out to detect the antibodies including immunobead test, mixed antiglobulin reaction (Coomb's), and Elisa estimation of antibodies. Direct immunobead test and mixed antiglobulin reactions are the most common tests performed [65]. The presence of more than 50% immunobead-reacted or mixed antiglobulin-reacted sperm is associated with

Table 12.2 Summary of conventional semen analysis and sperm function tests in varicocele

Test	Methods	Significance	Value in varicocele
Conventional semen analysis	WHO 2010 criteria	Semen parameters evaluate fertility potential.	Varicocele is associated with significantly reduced sperm count, motility, and morphology. Varicocelectomy improves semen parameters and is associated with higher pregnancy rate. .
Specialized sperm function tests			
AsAb	Immunobead test Mixed antiglobulin reaction (coomb's) Elisa estimation of antibodies	AsAb affects normal fertilization through interfering with sperm agglutination, cervical mucus penetration, and sperm–oocyte interaction.	AsAb has low positivity among clinical varicocele patients. Varicocelectomy's effect on AsAb titers is still controversial.
Vitality Assays	HOS test Eosin test Eosin–Nigrosin test	Used when sperm cells have low motility, lost their flagellation, have metabolic dysfunction or axonemal defects, or in case of necrozoospermia.	Varicocele is associated with sperm tail swelling. Varicocele repair improves sperm swelling in patients who achieve pregnancy. HOS test has poor prognostic value, due to high level of false-positive results.
Capacitation tests	Incubation in albumin-containing culture Computer-assisted sperm analysis (CASA) Chlortetracycline staining	Capacitation results in sperm with more fluid and pliable membranes, facilitating acrosome reaction.	Spermatozoa of varicocele patients have lower membrane fluidity caused, in part, by the higher peroxidative damage presented in their membrane.
Acrosomal integrity and function tests	Labeling with fluorescent lectins Monoclonal antibodies Histochemical staining Antibody-bound beads Flow cytometry	AR tests have high predictive power for the prediction of fertilization.	There is no significant association between varicocele and AR rates.
Zona binding assays	Hemizona assay Competitive intact zona binding assay	Both assays have high predictive value for in vitro fertilization results.	Both assays aren't capable of predicting who might benefit from varicocele repair. HZA results have a significant high correlation with pregnancy achievement after varicocelectomy.
Sperm penetration assay	Hamster Egg Penetration Test	Being an expensive and time-consuming test with unimproved reliability and reproducibility limits this test's ability to determine fertility potential.	Hamster zona-free oocytes penetrated by sperm cells are lower in varicocele men. Improvement in penetration after varicocelectomy is unclear.
Reactive oxygen species (ROS) estimation	Direct methods: Chemiluminescence Nitro blue tetrazolium test Flow cytometry Electron spin resonance Indirect methods: Endtz test Redox potential Isoprostane method	Provides an estimate of the state of seminal oxidative stress.	Oxidative stress markers are higher among varicocele patients. Oxidative stress has been implicated as a key element in the pathophysiology of varicocele-associated infertility. Varicocelectomy can reduce seminal and peripheral oxidative stress levels.

(continued)

Table 12.2 (continued)

Test	Methods	Significance	Value in varicocele
Spermatozoa DNA damage tests	Direct methods: Comet assay Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) nick end-labeling (TUNEL) assay Indirect methods: Sperm chromatin structure assay (SCSA) Sperm chromatin dispersion assay (SCD)	DNA damage in sperm cells has been linked to poor semen quality, impaired preimplantation development, and increased abortion.	Varicocele is associated with increased levels of SDF, inactive mitochondria, and abnormal chromatin packaging. High levels of SDF among varicocele patients are negatively associated with infertility. Varicocelectomy reduces SDF, increases sperm DNA integrity, and is associated with increased pregnancy rates.

subfertility, where the conception rate was significantly lower in immune couples compared to nonimmune couples [65, 66]. Also, the pregnancy outcome of immune couples was favorable only in 50% of the cases [66]. The detection of immunoglobulin type M (IgM) AsAb requires further investigation, as it might be an indication of recent trauma or testicular cancer.

Varicocelectomy effect on AsAb titers is still controversial. Some studies have revealed an almost 50% reduction in AsAb titers after varicocele repair, especially among patients with high-grade varicoceles [67, 68]. Moreover, in these studies, pregnancy within a year after varicocelectomy was 2.8 times more common in AsAb-negative than AsAb-positive men following surgery [67]. On the contrary, other studies have found that the reduction in AsAb titers does not predict improvements in semen parameters [62]. Based on the aforementioned data, AsAb testing in the clinical setting remains questionable.

Vitality Assays

Vitality tests are used when sperm cells have low motility, lost their flagellation, have metabolic dysfunction or axonemal defects, or in case of necrozoospermia (dead sperm). Vitality can be assessed by hypo-osmotic swelling (HOS) test, eosin test, or eosin–nigrosin test [69, 70].

HOS test was first introduced by Jeyendran et al. in 1984 [71]. This test is based on the permeability of intact membranes of the viable spermatozoa. Under hypo-osmotic conditions, cytoplasmic space of intact cells will swell and its tail will curl [72]. However, dead sperms with nonintact membrane are incapable of swelling in hypotonic media because of their leaky membrane [72]. Eosin test is based on the fact that eosin is excluded by live cells, so damaged cells will take up the eosin and are stained specifically pink. The nigrosin provides a dark background, which makes it easier to assess the slides that can be preserved for future assessment and record [69].

It is suggested that varicocele alters sperm membrane function, as men with varicocele were found to have significantly lower sperm tail swelling than men with idiopathic infertility [72, 73].

HOS test was shown to have a good potential in varicocele management evaluation, especially to predict pregnancy outcomes [74–76]. A significant improvement in sperm swelling was obtained after varicocele repair in patients who achieved pregnancy [74]. Still large-scale controlled studies are needed to explore these effects.

The results of HOS test correlate with other semen analysis parameters such as morphology and motility, but the data on its effects on fertility are not satisfactory [74].

In addition, this test has poor prognostic value, due to high level of false-positive results. Consequently, nowadays, the HOS test is mainly utilized as an additional indicator of sperm vitality and in cases of immotile cilia syndrome or severe asthenozoospermia.

Capacitation Tests

Capacitation is a time-dependent reversible process where loss of extrinsic proteins like acrosome-stabilizing factors and membrane cholesterol occurs [77]. This results in sperm with more fluid and pliable membranes, thereby facilitating acrosome reaction and inducing sperm hyperactivation [77]. Capacitation can be assessed by three different tests including incubation in albumin-containing culture, computer-assisted sperm analysis (CASA), and chlortetracycline staining [78, 79].

Incubation in albumin-containing culture is a very simple process that does not require an oocyte or mucus [78]. CASA distinguishes hyperactivated from nonhyperactivated sperm by the high curvilinear velocity, low linearity, and the large value of the amplitude of lateral head displacement of the former [80]. CASA allows objective and repeatable quantification of these patterns, as well [80]. Chlortetracycline staining allows detection by fluorescence microscopy, where acrosome-reacted sperm show a staining pattern different from that of capacitated sperm with intact acrosomes [81].

In a prospective study, spermatozoa from patients with Grade II and III varicocele and normozoospermic men were incubated in capacitating conditions for 6 hours [82]. Spermatozoa of varicocele patients showed a significant impairment to develop hyperactivated motility in comparison to normozoospermic men [82]. The incidence of sperm with phosphotyrosine immunoreactive tails evaluated by immunocytochemistry was significantly lower among varicocele patients, as well [82]. However, normozoospermic cells significantly increased their membrane fluidity in comparison to the spermatozoa from varicocele patients that had not shown an increase in their membrane fluidity during the incubation [82]. Hence, spermatozoa from patients with varicocele have an impairment to undergo capacitation-associated changes, such as protein tyrosine phosphorylation. This situation could be attributed to the lower membrane fluidity caused in part, by the higher peroxidative damage presented in their membrane.

Acrosomal Integrity and Function Tests

Acrosome reaction (AR) involves the release of lytic enzymes and exposure of membrane receptors, which will allow sperm penetration through the zona pellucida (ZP) and fusion with the oolema [83]. Acrosomal status integrity can be assessed by different methods including labeling with fluorescent lectins, monoclonal antibodies to specific proteins, histochemical staining, binding with antibody-bound beads, and flow cytometry [84]. AR can be impaired by the lack of an acrosome or acrosomal dysfunction [84]. However, acrosomal loss can be due to normal sperm death; hence, this test is often used in conjunction with a vitality test, such as the HOS test, to distinguish nonviable from reacted acrosomes [83, 84].

AR tests have high predictive power for the prediction of fertilization [85]. Semen samples with 5–30% of reacted spermatozoa have a higher fertility potential [85]. However, no significant association is observed between varicocele and the AR rates [86]. In response to stimulation with follicular fluid, AR rates are significantly lower in infertile patients; nonetheless, this finding does not appear to be influenced by varicocele [86]. Nevertheless, AR is currently used for research purposes or after IVF failure.

Unlike HOS test, acrosome reaction failed to show any benefit in varicocele patients. There was no significant difference found in the acrosome reacted spermatozoa in varicocele and nonvaricocele patients [87, 88].

Zona Binding Assays

The zona has a major role in controlling fertilization, as it is the only physiological inducer of the acrosome reaction [7, 89]. Abnormal sperm zona pellucida interaction may prevent successful fertilization. Zona binding is commonly evaluated by two assays: hemizona assay and a competitive intact zona binding assay [7, 89]. Despite having different methodologies, both assays have a similar primary endpoint that is tight binding of sperm to zona [66].

The hemizona assay utilizes human oocytes and their zona pellucida is isolated and divided into half [90]. One half is incubated with a fertile donor sperm and the other half is incubated with the

patient sperm [90]. Then, the ratio of fertile to donor binding, referred to as hemizona index HZI, is measured. A ratio of less than 30% is considered abnormal, since HZI of less than 30% is significantly associated with lower pregnancy rates after intrauterine insemination (IUI) treatment [91]. In competitive binding assay, both the patient and donor samples are labeled with different flurochromes and the binding rate (ratio of patient/donor sperm) is used to reflect the binding capacity [92].

Both assays have high predictive value for in vitro fertilization results, as they can detect cases likely to show failed or poor fertilization [85]. In oligozoospermic patients, 80% of sperm cannot normally bind to ZP [85]. The main utilization of these assays is among patients who have failed standard IVF, with limited utility in the setting of primary infertility. With the emergence of intracytoplasmic sperm injection (ICSI), these tests are no longer favored especially that treatments of these functional defects are still unavailable.

Despite the reported negative effects of varicocele on sperm fusion and penetration of the ZP, the ability of these tests to predict who might benefit from varicocele repair is still controversial [93, 94]. Sperm binding to the ZP, as measured by the hemizona assay, improved only in varicocele patients whose partners achieved pregnancy [93, 94].

HZA results had a significant high correlation with pregnancy achievement after varicocelectomy. This correlation was higher with HZA results than that of sperm cell parameters [93]. Sperm cell parameters improved among varicocele patients who underwent varicocele repair, with no correlation to conception. However, the HZA index improved only among varicocele-treated patients who achieved early pregnancies [93]. Despite such results that may suggest a good potential for HZA utility as a predictive test for those who may benefit from varicocelectomy, large-scale studies are still needed to accurately establish this fact.

Sperm Penetration Assay (Hamster Ovum Penetration Test)

The "hamster egg penetration test" (HEPT) or the "sperm penetration assay" (SPA) uses hamster ova to test sperm capacitation, acrosome reaction, fusion, and penetration through the oolemma and decondensation within the cytoplasm of hamster oocytes [95, 96].

The functional predictive value of this test is controversial owing to the difficulty in optimizing the test protocol, low standardization and reproducibility challenges, and high levels of false-negative results [97, 98]. A summary receiver operating characteristics (ROC) curve demonstrated that the sensitivity of SPA was only 37%, with a specificity of 95% [85]. Being an expensive and time-consuming test, with unproved reliability and reproducibility, limits this test's ability to determine fertility potential [97].

Varicocele alters gamete membrane fusion, which increases the clinical importance of sperm penetration assays [99]. It was shown that hamster zona-free oocytes penetrated by sperm cells were significantly lower in varicocele men as compared to infertile patients without varicocele [99]. However, studies exploring improvement in penetration after varicocelectomy are scarce with unclear results.

Reactive Oxygen Species (ROS) Estimation

Elevated OS is mostly reflected by excess ROS or lack of antioxidant-buffering capacity. Methods to estimate ROS can be broadly divided into two categories: direct and indirect assays [50].

Despite providing accurate results, direct ROS assays are expensive, which limits their clinical application [85]. Direct ROS assays include chemiluminescence (Fig. 12.1), nitro blue tetrazolium test, flow cytometry, and electron spin resonance [60, 85]. The most commonly used method to assess ROS concentration in semen is chemiluminescence [100]. However, leukocytes, cellular debris, analysis time, poor liquefaction, repeated centrifugation, changes in the pH, and other factors can affect the chemiluminescent reaction and its results. Nitro blue tetrazolium test is readily available, easily performed, inexpensive, and highly sensitive test [101]. Flow cytometry is expensive and requires skilled personnel and software for data analysis [102]. Electron spin resonance is also an expensive and

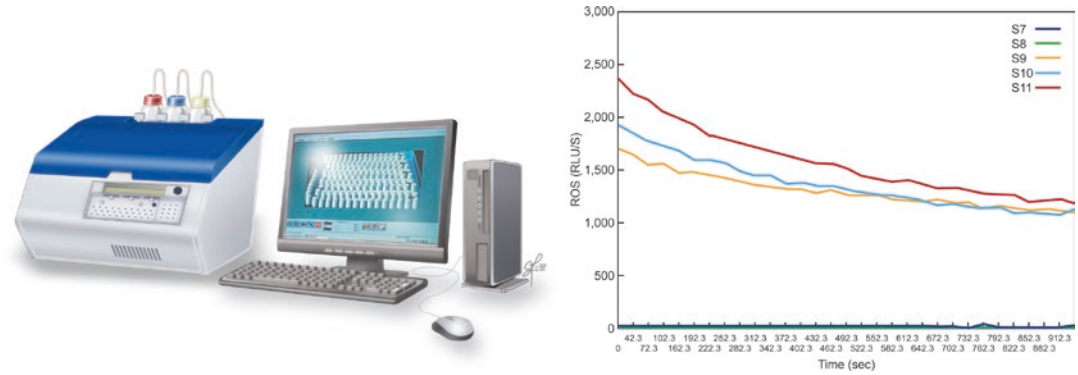

Fig. 12.1 Reactive oxygen species measurement by chemiluminescence assay: AutoLumat 953 Plus Luminometer connected to a computer with a sample result graph. This assay quantifies both intracellular and extracellular ROS using sensitive probes that react with oxidative end products, forming an electrical signal, which can be measured as counted photons per minute with a luminometer. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2011–2018. All Rights Reserved)

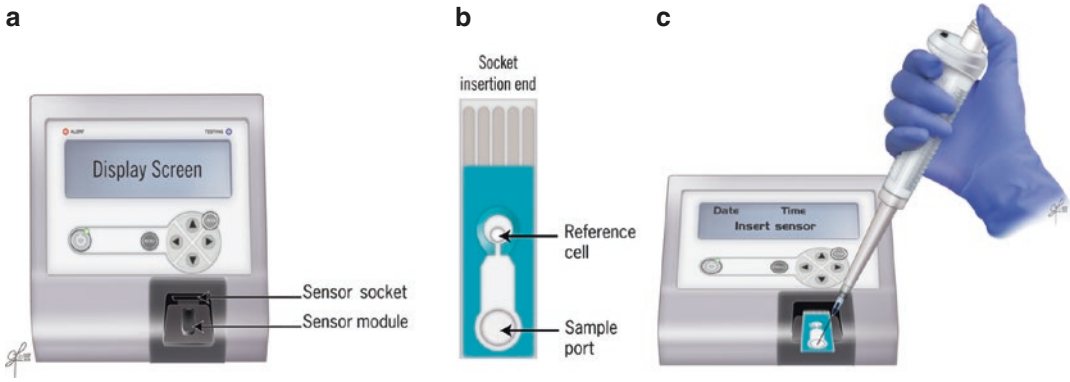

Fig. 12.2 The MiOXSYS system comprises of an (**a**) analyzer, (**b**) a disposable sensor, (**c**) application of the sample. Using a small semen sample, this system measures the oxidation–reduction potential level, which is an estimate of the balance between oxidants and reductants. (Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2011–2018. All Rights Reserved)

cumbersome technique that detects only selective oxidants, but is the most direct and rapid method for detecting free radicals with no effect from added chemicals [103].

Unlike the direct methods, indirect assays measure the stable end-products of the peroxidative process or DNA damage, and provide information of ROS-related damage [104]. Indirect assays include Endtz test, redox potential (Fig. 12.2), and isoprostane method [104].

Total antioxidant capacity (TAC) assesses the total antioxidant status of the semen. Catalase, glutathione peroxidase, and superoxide dismutase measurements provide an indirect assessment of seminal OS level [105]. The process is achieved by calorimeter or spectrophotometer [106]. TAC has low predictive value, limiting its use in infertility evaluation. Both ROS and TAC do not qualify alone to quantify seminal OS [107]. To improve their predictive value, a new index, known as ROS–TAC score, has been proposed [105–108]. A lower score in infertile men is significantly correlated with a higher risk of prolonged inability to conceive, and ROS–TAC scores below 30 affect negatively the fertility [105, 107, 108]. However, there is insufficient data available now to validate the use of this score in clinical practice.

OS markers are significantly elevated in varicocele patients. Seminal levels of nitric oxide, nitric oxide synthase, hydrogen peroxide, extracellular seminal superoxide anion, and malondialdehyde levels were found to be higher in infertile men with varicocele compared to fertile controls [109]. In addition, a direct relationship was observed between varicocele grade and seminal ROS levels [109, 110]. Such findings suggest the clinical usefulness for assessing ROS levels in men with varicocele. Oxidation–reduction potential, a recently developed method for rapid assessment of redox potential in semen samples, was found to be significantly higher in varicocele patients compared with normal controls [111, 112]. Additionally, the ORP result showed significant negative correlations with sperm concentration, motility, and normal morphology [111, 112]. OS is a potential target for therapeutic interventions, which helps clinicians to identify individuals most likely to benefit from such interventions and monitor the results.

Varicocelectomy can reduce seminal and peripheral oxidative stress levels in varicocele patients with a time lag of approximately 6 months [113–116]. Significant reduction in markers of seminal OS, including nitric oxide, hydrogen peroxide, and malondialdehyde, was reported [113, 116]. Whereas antioxidant levels of superoxide dismutase, catalase, glutathione peroxidase, and ascorbic acid were increased after varicocele repair [114, 116]. There is a direct proportional association between varicocele grade on the one hand and seminal ROS levels and decrease in sperm concentration on the other. Varicocelectomy in men with clinical varicocele and high levels of seminal ROS resulted in a rapid decline in free radical levels within 1 month [115, 116]. By reducing the potential for ROS generation, varicocelectomy was found to improve the disposal of residual sperm cytoplasm by the testis and epididymis [117]. Varicocele repair reduces spermatozoa with decreased residual cytoplasm, and increases the proportion of motile spermatozoa and normal forms after 6 months of the procedure [114, 117]. Not only OS-associated infertility is alleviated by varicocele repair but also a protection against the progressive character of varicocele and its consequent upregulations of systemic OS was observed as well [116].

Sperm DNA Fragmentation Tests

DNA damage in sperm cells has been linked to poor semen quality, impaired preimplantation development, and increased abortion [61, 118–120]. Natural fertility and IUI outcomes are reduced among men with high percentage of spermatozoa with DNA damage. Elevated OS status has been directly related to SDF levels in men with varicocele, as well [121]. ROS can induce damage to both nuclear and mitochondrial DNAs, resulting in base modification, strand breaks, chromatin cross-links and apoptosis-like process, affecting maturation and nuclear protamination [121, 122]. High levels of SDF among varicocele patients can have detrimental effects on fertility potential, both natural and assisted [118]. Degraded sperm is a specific subpopulation of sperm with substantial nuclear DNA damage that is more prevalent among varicocele men [123]. Despite not being exclusive to varicocele patients, degraded sperm was significantly overrepresented in this group. DDSi, the fraction of degraded sperm in a population of sperm with DNA fragmentation, accurately identified patients with varicocele 94% of times [123].

DNA integrity tests mostly examine DNAs with single- and/or double-strand breaks. These techniques include either direct methods such as the comet assay, and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) nick end-labeling (TUNEL) assay or indirect methods using the sperm chromatin structure assay (SCSA) and sperm chromatin dispersion assay (SCD) [61, 118].

Comet assay analyzes and detects DNAs with single- and/or double-strand breaks in many cells informatively, but it is not suitable for rapid diagnosis, is labor-intensive, requires a software to analyze the results, and the DNA damage can be overestimated [124, 125]. Because of TUNEL high predictive value for pregnancy outcomes and commercial availability of the test kit, it is currently recommended for measuring sperm DNA fragmentation [125,

126]. SCSA measures the stability of spermatozoa chromatin. It is a fast and easy test but an expensive technique to set up, owing to the need for flow cytometry and specialized personnel [127, 128]. Moreover, it is limited by interobserver subjectivity and rapid fading of the fluorescence [125, 127]. Despite allowing the analysis of large number of sperm, SCSA does not provide much information about the amount of DNA damage in a single sperm [127, 128]. The sperm chromatin dispersion (halo) test as well can easily detect the number of DNAs with both single- and/or double-strand breaks, but for a single spermatozoon [125, 129]. Similar to Comet assay, the setup is not easy, a dedicated software is needed, and DNA damage is usually overestimated [125, 129]. Unlike the other techniques, SCD assay is relatively inexpensive, rapid to use, and can simultaneously detect DNA and protein damages [129].

It is sometimes difficult to compare the results of these tests due to inadequate standardization of some of these methods and the cost to perform these tests. Different methods utilized may reveal different types of DNA breaks. However, all these tests can provide reliable results if internal controls are available and proper standardization is conducted [130].

Varicocele is associated with increased levels of SDF, inactive mitochondria, and abnormal chromatin packaging [131, 132]. Higher sperm DNA damage in varicocele men than controls was revealed, with a mean difference of 9.84% [132–134]. Moreover, high levels of SDF were detected in varicocele patients whose semen parameters fall within the normal reference ranges [132]. DNA damage induced by oxidative stress in varicocele patients increases the levels of abnormal mitochondrial membrane potential, and consequently increased levels of sperm with inactive mitochondria and early apoptosis levels [132]. Varicocelectomy reduces SDF with a mean difference of −3.37% compared to no treatment, improves sperm DNA integrity postoperatively, and is associated with increased pregnancy rates [135, 136]. However, surgical ligation procedure reduced the SDF in clinical but not in subclinical varicoceles [18].

Abnormal chromatin packaging level is also elevated in the semen of patients with varicocele [137, 138]. Sperm chromatin packaging reduces the ability of spermatozoa to fertilize oocyte [127]. The rate of chromatin condensation using aniline blue staining in infertile men with varicocele significantly improved following surgical correction of large varicose veins [137]. Varicocele repair results in rapid decline in free radical levels after 1 month of the procedure followed by a slow decline in SDF assessed by the Comet assay after 3–6 months [137].

The acridine orange (AO) assay measures the ability of sperm nuclear DNA to denature by acid, where AO fluorescence shifts from green with native DNA to red with denatured DNA [139]. Semen analysis using AO staining can be performed in a clinician's office with a fluorescent microscope. After varicocele repair, AO test provided significant results compared to flow cytometry that correlated with pregnancy outcome [139]. This is a simple, reliable, rapid, and cheap test for DNA integrity evaluation test in basic andrology laboratories. However, it still requires validation with more extensively used and well-known methods.

The extent of research conducted on SDF has triggered the editors of this book to formulate guidelines on the utility of SDF in clinical practice, which were endorsed by the Society of Translational Medicine [140]. Among the various clinical indications for SDF testing, the committee recommends its utility in varicocele patients as the test result may allow clinicians to better select varicocelectomy candidates.

Emerging Technologies

Novel technologies are emerging from andrology research laboratories. These technologies may soon become available for clinical utility.

One of the most important novel techniques are the –omics technologies, which study genes (epigenomics and genomics), transcripts (transcriptomics), proteins (proteomics), and metabolites (metabolomics) [141, 142]. These OMICS technologies study the interactions of cellular

structures and processes from DNA to biological function: from DNA and genes to metabolites in a complex and global way [142]. Inventories of lipid, proteins, metabolites, and RNA species can be determined with these technologies; this can provide insights into the biochemical basis of defective semen quality [143, 144].

One potential diagnostic method for evaluating male factor infertility is microarray, which studies spermatozoal RNA profiles that provide a historical record of spermatogenesis [145]. Microarray analysis of spermatozoal mRNAs in fertile and infertile men with normal semen parameters showed a profound differential expression of hundreds of genes between both groups [146]. Hence, this technology might be used in the future to investigate the response of sperm cells to changes in the environment or conditions that alter mRNA expression, allowing insight into the mechanisms of diseases on fertility. Preliminary studies have shown an altered genomic expression pattern in the spermatozoa of infertile men.

Seminal fluid has more than a thousand different proteins, making it an appealing specimen for proteomic analysis [147]. At least 20 seminal proteins have had altered expressions in infertile men [147]. Proteomic analysis at this time is mainly dedicated to identifying key seminal proteins, such as fibronectin, lactoferrin, laminin, albumin, semenogelin, heat shock protein 2, and sperm acrosome membrane–associated protein [125]. This field is of extreme importance in varicocele patients who may have their fertility compromised even though their semen analysis parameters are within reference ranges: this can be explained by the disturbance of the spermatozoa at the molecular level [148]. For instance, nitric oxide metabolism was found to be activated in patients with varicocele [149], whereas varicocelectomy increased the expression of different proteins, including superoxide dismutase 1 (SOD1) and ATP synthase, H+ transporting, mitochondrial F1 complex, and delta subunit (ATP5D) in spermatozoa [150, 151]. Seminal plasma proteins including calcium-binding protein (CAB45) and cysteine-rich secretory protein 3 (CRISP3) also showed altered expressions in patients with varicocele [149].

Comparative proteomic analysis identified 58 differentially expressed proteins (DEPs) in bilateral varicocele and 38 unique proteins in unilateral varicocele [152–154]. It was predicted that glutathione S-transferase mu 3 (GSTM3), sperm protein associated with the nucleus, X chromosome, family member B1 (SPANXB1), Parkinson disease protein 7 (PARK7), proteasome subunit a8 (PSMA8), dihydrolipoamide dehydrogenase (DLD), SEMG1, and SEMG2 are potential biomarkers to differentiate unilateral from bilateral varicoceles [153].

Proteomic studies on ROS-positive and -negative varicocele patients produced several potential biomarkers in both spermatozoa and seminal plasma [155]. Important proteins such as fibronectin 1 (FN1) and macrophage migration inhibitory factor (MIF) were absent in the ROS-positive group, while membrane metalloendopeptidase (MME) protein was absent in the ROS-negative group [155].

Genetic alteration has tremendous effects on the formation and function of the genitourinary systems. Chromosomal abnormalities occur 15 times more among infertile men as compared to the general population [156, 157]. Genetic mutations and polymorphisms were recognized in infertile men, even when spermiograms were normal [158]. Testing for genetic conditions is recommended when infertility in men with less than 5 million total motile sperm could be related to gene deletions, mutations, or chromosomal abnormalities [159]. In addition, azoospermia or severe oligozoospermia cases could be induced by deletions in the Y-chromosome, known as the DAZ gene [160, 161]. The karyotype is normal (46 XY), but further evaluation of the Y-chromosome shows some missing sections of this Y-chromosome [161].

Conclusion

Varicocele is a complex clinical condition that can carry detrimental effects on male fertility. Nonetheless, a good number of men with varicocele are still capable of natural conception or may have normal conventional semen parameters. Such a finding has elicited the utility of advanced

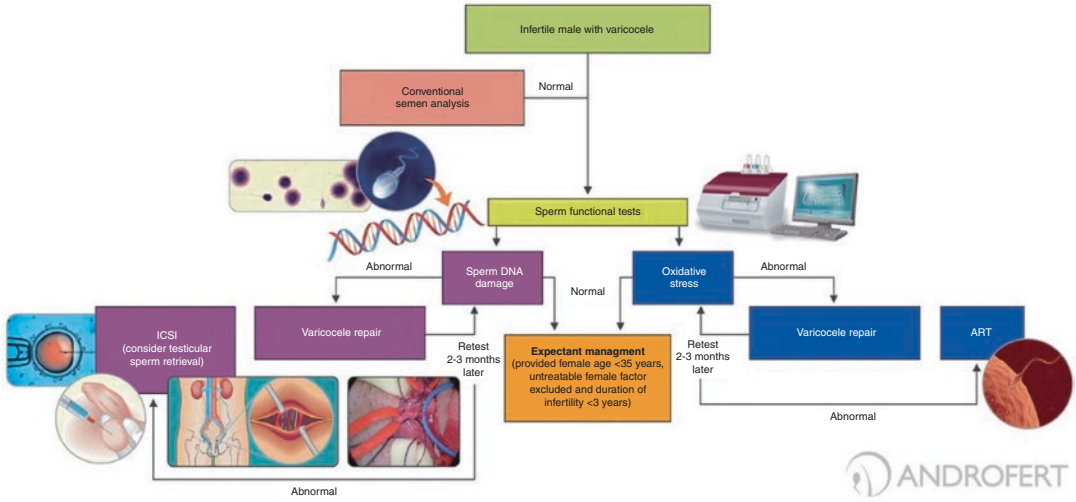

Fig. 12.3 Algorithm proposed for the management of infertile males with varicocele using sperm DNA damage and oxidative stress tests. Testing is recommended at initial workup to all men with conventional semen analysis results within normal ranges. Abnormal test results identify couples at higher risk of remaining childless if an expectant management is taken. Interventions aimed to overcome OS and sperm DNA damage in patients with varicocele include varicocele repair and assisted reproductive techniques. Oral antioxidants and life-style modifications (cessation of smoking, weight loss) can be combined to varicocelectomy. Monitoring is carried out with same tests at 3-month intervals after varicocele treatment. ART is recommended for patients with persistent abnormal sperm function markers after varicocele repair. Testicular spermatozoa may be considered for sperm injections in ART treatment. (Reprinted with permission, ANDROFERT© 2018. All Rights Reserved)

sperm function tests for the evaluation of varicocele patients, which is still hindered by standardization issues and/or cost. Therefore, large-scale, well-designed, and controlled studies are essential to clarify the association of sperm function tests with fertility potential in varicocele patients and to identify who may benefit most from varicocele repair. Of all the sperm function tests available, measures of OS and SDF are perhaps most commonly utilized with available algorithms directing their use in varicocele patients (Fig. 12.3). Future studies will wide open the door for emerging technologies to prove their efficacy as promising diagnostic or prognostic tools among infertile men, especially varicocele patients.

Review Criteria

An extensive search of the literature was done using scientific search engines including PubMed, Medline, ScienceDirect, and Google Scholar. Search criteria included the following key words: "varicocele," "semen analysis," "sperm parameters," "oxidative stress," "sperm DNA fragmentation," and "sperm function tests." Data from published papers or book chapters were included.

Multiple Choice Questions and Answers

1. The WHO 2010 manual for conventional semen analysis
   (a) caused confusion among clinicians and resulted in over- or underdiagnosis
   (b) defined values from data that were derived from imprecisely defined normal reference populations
   (c) is the 6th edition of a series of manuals describing this laboratory test

(d) **was based on semen values of fertile men who became fathers with a time to pregnancy of less than 12 months**
(e) proposed reference values based on the tenth centile of the lower cutoff limits

2. Which of the following statements about conventional semen analysis in the context of varicocele is FALSE?
   (a) The exact mechanisms behind the effects of varicocele on semen quality remain uncertain.
   (b) Fertile and infertile men with varicocele may have similar semen parameters to those without varicocele.
   (c) Varicocele was associated with significantly reduced sperm count, motility, and morphology but not semen volume.
   (d) **A direct relationship is found between the varicocele grade and the semen analysis result.**
   (e) In Grade 3 varicocele, sperm concentration is usually less than half of that in men with no varicocele.
3. Regarding varicocelectomy, the following statement is FALSE:
   (a) Have been found by few studies to not have a statistically significant effect on semen parameters or pregnancy rates.
   (b) The maximal effect can be observed 3 months after surgery.
   (c) Significant improvements in sperm count and motility are observed regardless of the chosen surgical technique.
   (d) **The highest increment in sperm count and sperm motility was achieved by the retroperitoneal approach, with no clinical significance when compared to other techniques.**
   (e) Varicocelectomy improves sperm motility and concentration in all varicocele grades, but mostly among Grade 3 patients and may take a little longer than 3 months.
4. Which of the following sperm function tests are most informative during the evaluation of infertile men with varicocele?
   (a) AsAb and vitality assays
   (b) Capacitation and acrosome reaction tests
   (c) Zona binding assays and sperm penetration assays
   (d) **Oxidative stress markers and sperm DNA fragmentation**
   (e) All of the above
5. Which of the following statements is/are true?
   (a) The extensive research on sperm DNA fragmentation revealed its uselessness in the evaluation of men with varicocele.
   (b) No relationship between seminal oxidative stress and sperm DNA fragmentation has been observed.
   (c) **Society-endorsed guidelines for the utility of sperm DNA fragmentation in clinical practice have been published.**
   (d) Varicocelectomy was not associated with a decline in oxidative stress measures postoperatively.
   (e) Seminal levels of nitric oxide, nitric oxide synthase, hydrogen peroxide, extracellular seminal superoxide anion, and malondialdehyde levels were found to be lower in infertile men with varicocele compared to fertile controls.

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13 Endocrine Testing and the Association Between Varicocele and Hypogonadism

Grace Yaguchi and Ali A. Dabaja

Key Points

- Varicocele results in progressive duration-dependent injury to the testicle including spermatogenesis and reversible Leydig cell dysfunction.
- Endocrine testing suggested as initial laboratory screen for male reproductive dysfunction includes at a minimum total testosterone, sex hormone–binding globulin, albumin, LH, FSH, and estradiol.
- Microsurgical varicocele repair is associated with increased serum testosterone levels in the hypogonadal male.

Introduction

Varicocele results from an abnormal dilation of the pampiniform vein plexus within the spermatic cord. Varicoceles are extremely common, affecting 14–25% of males overall, but they do not cause infertility in most affected men. However, among men that present to infertility clinics, 35–60% are diagnosed with a varicocele [1].

Surgical correction of varicocele, via varicocelectomy or embolization, has been recommended to improve semen parameters and varicocele is the most common reversible factor in male infertility. The negative effects of varicocele on spermatogenesis are well-proven by measurable improvements in semen parameters after surgical correction. However, the pathophysiology of the varicocele insult to spermatogenesis is not completely understood. It does appear that varicocele results in progressive testicular injury and impacts spermatogenesis. Additionally, increasing research studies and analysis indicate that correction of varicocele may also improve the function of Leydig cells, leading to improvement of testosterone levels in the hypogonadal male. Hypogonadism results from failure to produce adequate concentrations of serum testosterone, normal amounts of sperm, or both. The presentation of hypogonadism varies but can include erectile dysfunction, decreased sex drive, loss of muscle mass, and loss of bone composition. Hypogonadism is also associated with male infertility; thus, endocrine testing is an important component in the evaluation of the infertile men. Improvement in testosterone levels may represent an additional quantitative end-point for varicocele correction surgery and hypogonadism may be considered an indication for varicocele repair in the future. This chapter will examine the effects of varicocele on serum testosterone levels.

G. Yaguchi · A. A. Dabaja (✉)
Vattikuti Urology Institute, Henry Ford Hospital, Detroit, MI, USA
e-mail: adabaja2@hfhs.org

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Male Pituitary–Gonadal Axis and Hypogonadism

Male Pituitary–Gonadal Axis Overview

To better understand the impact of varicocele on the testicle and the endocrine function of testosterone production, a brief overview of the male hypothalamic–pituitary–gonadal (HPG) axis and testosterone production will be reviewed (Fig. 13.1).

Hypothalamus

The hypothalamus is the center of the HPG axis. It coordinates the body's response and influences a variety of important functions including food and energy homeostasis, fluid balance, body temperature, and the sleep cycle. Most of these responses are in concert with pituitary function and hormone release. The hypothalamus is anatomically linked to the pituitary gland by a portal vascular system and neuronal pathways. It can therefore directly deliver the hypothalamic hor-

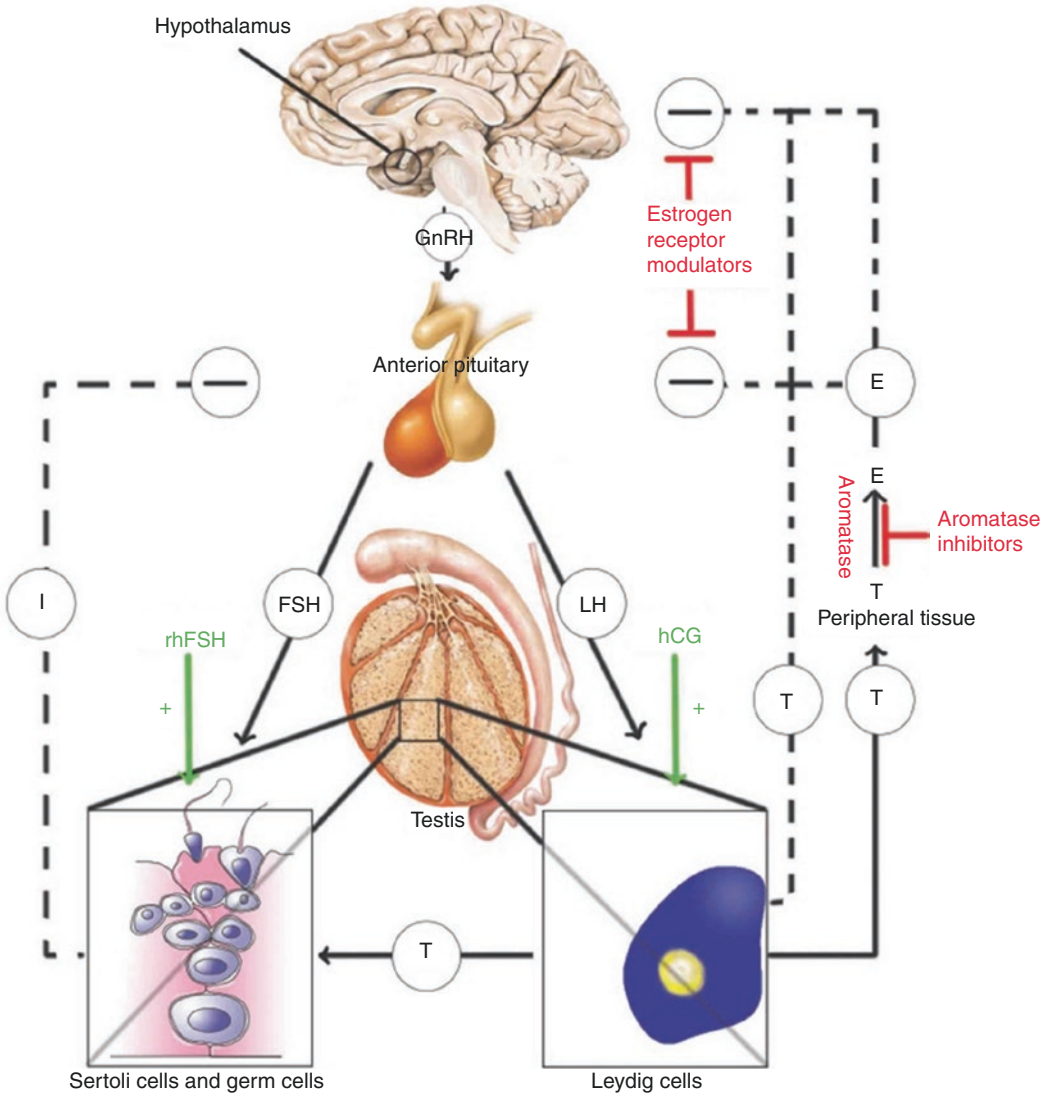

Fig. 13.1 Male hypothalamus–pituitary–gonadal axis. (Illustration created and provided courtesy of Vanessa Dudley)

mones and avoid systemic circulation when signaling for the release of pituitary hormones. The hypothalamus releases many hormones, but the most important for reproduction is gonadotropin-releasing hormone (GnRH), which functions to stimulate the secretion of luteinizing hormone (LH) and follicular-secreting hormone (FSH) from the anterior pituitary [2].

Anterior Pituitary

The anterior pituitary is regulated by the hypothalamus and releases various hormones into systemic circulation, where they produce a physiologic response in their respective target organs. The release of these hormones is cyclic with the rhythms driven by the nervous system. This cyclic release is not well understood, but is important in maintaining homeostasis.

After its secretion from the hypothalamus, the GnRH is carried via the portal vascular system to the anterior pituitary where it stimulates the production and release of LH and FSH. LH and FSH are only known to act on the gonads, stimulating spermatogenesis and testosterone hormone production. The anterior pituitary also produces prolactin, which, at high levels, can interfere with the episodic GnRH release by the hypothalamus, thus resulting in decreased LH and FSH production [2]. These hormone signals act in concert; pulsatile GnRH causes pulsatile LH release, which stimulates the testosterone production in a pulsatile manner as well. This is important to maintaining circulating levels of testosterone, as continuous testosterone infusions lead to greater suppression of testosterone by LH [3]. The circadian rhythm of testosterone levels is more pronounced in younger men with peak levels in the morning and trough in the afternoon. As men age, the rhythm and concentration of testosterone decrease [4].

Gonad

The male gonad, the testis, has numerous exocrine and endocrine functions, which contribute to normal male virility and fertility. The Leydig cells, found within the interstitial compartment of the testis, are responsible for steroidogenesis. LH stimulates steroidogenesis in the Leydig cells by inducing hormone synthesis via conversion of cholesterol to testosterone within the mitochondria. FSH binds to Sertoli cells and is the major stimulator of quantitatively normal levels of spermatogenesis in the adult male. Negative feedback hormones are also produced by the testis. Inhibin is stimulated by FSH and acts as negative feedback at the pituitary or hypothalamus. Testosterone acts on androgen receptors to provide negative feedback suppression mainly at the hypothalamus [2].

Testosterone

During development, in utero through adolescence, testosterone is the male hormone affecting development and maturation of the male reproductive system. In adulthood, testosterone is integral for the maintenance of fertility, and exerts overall anabolic effects on muscle and bone. In bone, testosterone increases osteoblast number and lifespan. This acts to reduce bone resorption. Testosterone also influences lipid uptake and activity of adipocytes.

Intratesticular testosterone concentrations in eugonadal men are 100-times greater than serum levels of testosterone [5], which is compatible with the Leydig cell production of testosterone within the testis. Testosterone plays an important role in Sertoli cell function regulation and the orchestration of spermatogenesis. When intratesticular testosterone concentration decreases, spermatogenesis by Sertoli cells also decreases [6].

When circulating in the blood stream, the majority of testosterone is bound to sex hormone–binding globulin (SHBG) and is biochemically unavailable for the functions described earlier [2].

Hypogonadism

Testosterone serves important roles in male development and maintenance of virility. In utero, the development of male genital organs is dependent on testosterone. In the adolescent, testosterone is important for the initiation of spermatogenesis and development of secondary

sexual characteristics. In adulthood, testosterone is the predominant circulating androgen in males. Androgen deficiency is associated with a decrease in sexual function, as well as metabolic and musculoskeletal complications. These complications include decrease in muscle mass, muscle strength, and reduction of bone mineral density.

Primary hypogonadism is a result of testicular failure, while secondary hypogonadism is defined as disruption of the HPG axis described earlier. Defects at the central level, within the HPG axis, could be due to pituitary pathology.

As men age, a progressive decline in serum levels of testosterone, both free and total, is observed. Recent studies have shown that the calculated free testosterone levels correlate with hypogonadism symptoms as men age than the total testosterone level [7]. The concentrations of circulating LH do not decline with age, suggesting that primary gonadal hypofunction, rather than changes within the hypothalamic–pituitary axis, is responsible for the reduced testosterone levels. The reduced testosterone levels may be due to a decrease in the number of Leydig cells or the functional androgenic activity of those cells. Although animal models and studies on the use of human chorionic gonadotropin (hCG) indicate that the decline is due more to reduced function while the overall number of Leydig cells remains relatively unchanged with age.

In addition to age, testicular injury and subsequent decrease in circulating testosterone have been demonstrated secondary to systemic illness such as AIDS, end-stage renal disease, liver disease, chronic opioid use, and cytotoxic damage from radiation or chemotherapy. The pathophysiologic mechanisms for the decrease in circulating testosterone observed in men with these comorbid conditions and diseases are varied. They include cytotoxic damage, testicular atrophy due to nonspecific interstitial inflammation, testicular fibrosis secondary to opportunistic infections, and defects in Leydig cell morphology [8]. These well-documented disease sequelae and the adverse effect on testosterone production may offer insight into how the varicocele changes the testicular microenvironment and can contribute to hypogonadism. While varicocele is not a multisystem disease process like those listed earlier, the varicocele can also contribute to hypogonadism by altering the testicular environment with the same pathophysiologic effects as described for other systemic illnesses.

Endocrine Testing

Screening Versus Diagnosis of Hypogonadism/Androgen Deficiency

Men may present to the clinician with complaints consistent with androgen deficiency. Unfortunately, the signs and symptoms of androgen deficiency are nonspecific and can have variable presentation based on factors such as age, comorbid illnesses, and variation in androgen sensitivity.

There are several validated surveys and tools that can be used by the clinician to screen for androgen deficiency [9]. However, these screening surveys lack specificity for androgen deficiency, making their usefulness in diagnosis and follow-up of treatment difficult to determine [10]. After screening, men with suspicious signs or symptoms of androgen deficiency will need biochemical testing for confirmation before making a diagnosis. Regrettably, there is no defined threshold serum testosterone concentration for symptoms of androgen deficiency and adverse outcomes. Various international societies of Endocrinology [11], Urology [12], and Andrology [13] have different definitions of total testosterone concentration that qualifies as androgen deficiency. This variation in definition of the total testosterone concentration in biochemical hypogonadism, as well as the lack of tool for evaluating treatment efficacy, should be considered when evaluating patients for the diagnosis and treatment of hypogonadism.

Other Clinical Indications for Endocrine Testing

All men presenting for evaluation of infertility should undergo history, physical examination, and laboratory testing. The laboratory assess-

ment generally includes endocrine evaluation, semen analysis, and genetic testing. The endocrine testing suggested as initial laboratory screen for male reproductive dysfunction includes total testosterone, sex hormone–binding globulin, albumin (for calculation of bioavailable testosterone), LH and FSH (for pituitary function), prolactin, and estradiol (for aromatization).

Total Testosterone

The threshold of serum total testosterone that would indicate low testosterone or hypogonadism is not universally established; however, serum total testosterone <350 ng/dl is considered a reasonable threshold for diagnosis of testosterone deficiency in the symptomatic patient by the American Urologic Association [14]. Total testosterone concentration can be confounded by sex hormone–binding globulin (see the following section).

In patients less than 40 years old, the blood testing for testosterone should be performed in the morning due to the diurnal variation in testosterone levels [15]. These diurnal variances are muted as men age, which makes the timing of the testosterone blood test less important [16].

Free Testosterone

Free testosterone represents biochemically available testosterone. The majority of testosterone circulating in the blood stream is bound to sex hormone–binding globulin and is unavailable for biochemical functions when in this bound state [2]. Free testosterone values are clinically useful in patients who have the symptoms consistent with testosterone deficiency and yet have a total testosterone within the normal range. Free testosterone reference ranges vary based on the laboratory and are not clinically based. Free testosterone can be measured directly with radioimmunoassay or calculated from total testosterone and sex hormone–binding globulin concentrations [17].

Sex Hormone–Binding Globulin and Albumin

These values are used to calculate the free testosterone [18]. Circulating testosterone is nonspecifically bound to albumin, specifically bound to sex hormone–binding globulin (SHBG), and also unbound, or free. Laboratory calculators have been developed using SHBG and albumin to calculate the free testosterone in a linear fashion. Albumin-bound testosterone equals the product of the association constant of albumin, albumin concentration, and free testosterone fraction [19].

LH and FSH

LH and FSH should be tested in patients with low testosterone to offer insight into the pituitary function. These hormones will be significantly increased in primary testicular failure. For other etiologies, these lab values will be normal or low. FSH may assist in the evaluation of hypogonadism/androgen deficiency, especially where fertility is a concern. While FSH does not stimulate production of testosterone (see the preceding section), it is considered a more sensitive indicator of testicular insufficiency [18].

Prolactin

Prolactin is recommended in the initial evaluation of low testosterone to rule out prolactinoma or prolactin-secreting tumor as a secondary cause of hypogonadism. If prolactin levels are elevated, an MRI should be performed to rule out pituitary adenoma [18].

Estradiol

Elevated estradiol levels can indicate increased aromatization of testosterone by aromatase. As described earlier, estradiol inhibits GnRH and LH secretion by negative feedback at the level of both the hypothalamus and pituitary (see Fig. 13.1).

Inhibin B

Inhibin B has been demonstrated as an important part of the male pituitary-gondal axis, as described in this chapter. It may prove to be a useful tool in assessment of testicular function, especially when combined with FSH assay. However, reference ranges with respect to the spermogram are still being developed [20].

The initial laboratory screening tests can be used to begin to differentiate between causes of infertility. The association between varicocele

Table 13.1 Initial endocrine test results and clinical conditions causing infertility

Clinical condition	FSH	LH	Testosterone	Prolactin
Normal spermatogenesis	Normal	Normal	Normal	Normal
Hypogonadotropic hypogonadism	Low	Low	Low	Normal
Abnormal spermatogenesis	High/normal	Normal	Normal	Normal
Complete testicular failure/Hypergonadotropic hypogonadism	High	High	Normal/low	Normal
Prolactin-secreting pituitary tumor	Normal/low	Normal/low	Low	High

and hypogonadism discussed in this chapter would be considered under the category of hypogonadotropic hypogonadism. Equivocal test results should be repeated [21] (Table 13.1).

Varicocele and Hypogonadism

Varicocele Effect on the Testicular Endocrine Function

The mechanisms of varicocele effects on the testicle remain unclear. These effects may be mediated by increased intratesticular temperature, increased oxidative stress, and the reflux of gonadotoxic renal and adrenal metabolites [22]. While these alterations to the testicular microenvironment have been postulated as impacting spermatogenesis, there is increasing evidence that other functions of the testicle, namely, the production of testosterone by Leydig cells and the delivery of this hormone to the systemic blood stream, may also be impacted by varicocele. Testicular biopsy in patients with varicocele demonstrates decreased tubular diameter, and increased Leydig cell atrophy [23]. Increased testicular temperature is associated with Leydig cell apoptosis and atrophy in humans. This elevated testicular temperature can inhibit 17a-hydroxyprogesterone aldolase, an enzyme necessary for the conversion of 17a-hydroxyprogesterone into testosterone [24]. Animal models were initially used to investigate the relationship among varicocele, testosterone levels, and Leydig cell function [25]. In humans, men with varicoceles have lower testosterone levels than a comparison group without clinical varicoceles [26, 27].

The testicular pampiniform plexus facilitates the exchange of heat and small molecules. Testosterone moves in a concentration-limited manner from veins to the artery via passive diffusion within the vascular arrangement of the pampiniform plexus [2]. The veins of the pampiniform plexus have thin walls, which contributes to the diffusion of heat and testosterone. Thus, this diffusion and the delivery of testosterone to the arterial blood supply may be impacted in men with varicocele. A recent study by Han et al. sought to compare the testosterone concentration in the peripheral blood and the spermatic vein plexus. They reason the hydrostatic pressure in the spermatic vein is not related to the diameter of the cord, but rather the height of the vertical vessels. This increased hydrostatic pressure causes disorder in the distribution of the testosterone to the peripheral blood of the varicocele patient [28].

Additionally, the increase in temperature within the scrotum is known to cause increase in reactive oxygen species [29]. The Leydig cell may also be more susceptible to these reactive oxygen species due to their proximity to interstitial macrophages, a principal contributor to endogenous reactive oxygen species [30].

Varicocele Repair to Treat Hypogonadism

Surgical correction of varicocele has been performed in the past to treat infertile men and varicocele-related pain. The increasing evidence linking varicocele with Leydig cell dysfunction has amplified interest in expanding the indications for surgical repair of clinical varicocele to include hypogonadism [31]. The current treatment for primary hypogonadism includes testosterone replacement therapy or use of GnRH agonist. Exogenous testosterone replacement has

been linked with serious side effects and risks including increased thrombotic events, cardiovascular events, and prostate cancer [17, 32]. While the risks of treatment with testosterone therapy continue to be investigated, varicocele repair to treat hypogonadism would presumably avoid unnecessary increase in relative risk, because it improves the function of Leydig cells while maintaining the body's endocrine feedback system for homeostasis [24].

Studies have been conducted on animal models and induced varicocele followed by surgical correction. These animal models demonstrate decreased intratesticular testosterone levels with an induced varicocele. The testosterone levels improve with repair of the varicocele, but not to the prevaricocele testosterone levels. However, these studies are based on acute surgical induction of varicocele and repair within 4–8 weeks. In humans, this acute presentation and early repair of varicocele does not commonly occur [25].

Several studies in the last 25 years have measured testosterone levels before and after varicocele repair. The early retrospective studies were mixed when measuring testosterone levels as a secondary endpoint in both eugonadal and hypogonadal men [27]. More recent studies have more closely evaluated infertile men with low or low-normal total testosterone levels undergoing varicocele repair surgery [33]. Varicocele repair in these men did demonstrate improvement in total testosterone. The improvement in serum testosterone levels following varicocele repair is demonstrated in men across age groups (16–65 years old) who have a pre-operative testosterone <400 mg/dL [34]. However, the long-term effect of varicocele on testosterone production is unknown and the use of endocrine testing is not a standard part of the evaluation of adolescent varicocele. Thus long-term effects of varicocele repair on future development of low testosterone and clinical hypogonadal symptoms are unknown [35]. The studies that have demonstrated an improvement in serum total testosterone employed magnification, with microsurgical varicocele repair showing the most consistent postoperative improvement in serum testosterone [36] (Table 13.2).

Table 13.2 Human studies on the effect of varicocelectomy on testosterone

First author, year	Study design	Number treated for varicocele	Intervention	Baseline testosterone (ng/dL)	Postoperative testosterone (ng/dL)	Change (ng/dL)	*P* value
Jangkhah et al. (2018)	Prospective	115	Microsurgical	567	594	27	0.05
Gomaa et al. (2018)	Prospective	45	Loupe-assisted subinguinal	490	660	170	0.0001
McCullough et al. (2017)	Retrospective	214	Robotic-assisted microscopic	327	472	145	0.0001
Vyas et al. (2017)	Prospective						
		30	Open subinguinal	550	631	81	
		30	Loupe-assisted subinguinal	549	107.6	508.6	
Naraji et al. (2017)	Retrospective	20	Microsurgical	379	536	136	0.139
Shabana et al. (2015)	Prospective	123	Microscopic subinguinal	385	447	62	0.0001
Ahmed et al. (2015)	Prospective	73	Microsurgical subinguinal	331	357	26	0.001
Abdel-Meguid et al. (2014)	Prospective	66	Microsurgical subinguinal	347	392	45	0.0001

(continued)

Table 13.2 (continued)

First author, year	Study design	Number treated for varicocele	Intervention	Baseline testosterone (ng/dL)	Postoperative testosterone (ng/dL)	Change (ng/dL)	*P* value
Hsiao et al. (2013)	Retrospective	78	Microsurgical subinguinal	308	417	109	0.0001
Hsiao et al. (2011)	Retrospective	—	Microsurgical subinguinal	—	—	—	—
	Age < 30	31		NA	NA	93	0.03
	Age 30–39	55		NA	NA	59	0.02
	Age > 40	28		NA	NA	73	0.001
Sathya Srini and Belur Veerachari (2011)	Prospective	100		177	301	124	0.001
Tanrikut et al. (2011)	Retrospective	200		358	454	96	0.001
Zohdy et al. (2011)	Prospective	103		379	450	71	0.0001
Resorlu et al. (2010)	Retrospective	—	Microsurgical subinguinal	—	—	—	—
	Age 18–25	35		275	297	22	>0.05
	Age 26–35	43		290	306	16	>0.05
	Age > 36	18		274	291	17	>0.05
Rodriguez-Peña et al. (2009)	Retrospective	202	Inguinal	648	709	61	>0.05
Ozden et al. (2008)	Prospective	30	Subinguinal	660	720	60	0.1
Di Bisceglie et al. 25 (2007)	Retrospective	38	ISV sclerotherapy	650	660	10	0.9
Hurtado de Catalfo et al. 51 (2007)	Retrospective	36	Not specified	298	382	84	NA
Gat et al. 19 (2004)	Retrospective	83	ISV embolization	348	497	149	0.001
Fujisawa et al. 70 (2001)	Retrospective	52		460	470	10	>0.05
Pierik et al. 20 (2001)	Retrospective	30		542	571	29	>0.05
Cayan et al. 18 (1999)	Retrospective	78		563	837	274	0.01
Su et al. 17 (1995)	Retrospective	53		319	409	90	0.001

Conclusion

Varicocele is a very common entity. There is increasing evidence that varicocele results in progressive duration-dependent injury to the testicle including spermatogenesis and reversible Leydig cell dysfunction. While surgical correction of varicocele has been performed in the past to treat infertile men and varicocele-related pain, there is increasing interest in performing varicocelectomy as a preservation of future infertility and prevention of Leydig cell dysfunction. There is evidence that varicocele repair can also result in increase in serum testosterone levels in hypogonadal men. With careful selection and counseling, microsurgical varicocele repair may be offered to men with clinical varicocele and hypogonadism to improve their total serum testosterone.

Review Criteria

An extensive search of studies examining the relationship between varicocele and hypogonadism was performed using search engines such as OVID, PubMed, and MEDLINE. The end dates for these searches were August 2018. The overall strategy for study identification and data extraction was based on the following key words: "varicocele," "hypogonadism," "testosterone," and "varicocelectomy." Articles published in languages other than English were not considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included. Websites and book-chapter citations provide conceptual content only.

Multiple Choice Questions and Answers

1. In the normal hypothalamic–pituitary–gonadal axis for men, inhibin acts as negative feedback at the pituitary or hypothalamus. What stimulates the release of inhibin?
   (a) At high levels, inhibin released by the anterior pituitary can interfere with the episodic GnRH release by the hypothalamus.
   (b) **FSH stimulates the release of inhibin by the testicle.**
   (c) GnRH is carried via the portal vascular system to stimulate the production and release of inhibin by the anterior pituitary.
   (d) Testosterone stimulates the release of inhibin by muscle cells.
2. What is the pathophysiology believed to explain the observed association between varicocele and hypogonadism?
   (a) **The elevated testicular temperature can inhibit 17a-hydroxyprogesterone aldolase, an enzyme necessary for the conversion of 17a-hydroxyprogesterone into testosterone.**
   (b) The decreased tubular diameter demonstrated in testicular biopsy in patients with varicocele results in a large decrease in tubular volume. This results in a decrease in the conversion of 17a-hydroxyprogesterone into testosterone.
   (c) The varicocele increases the production of the androgen-binding protein, sex hormone–binding globulin (SHBG), making less testosterone bioavailable for initiating protein synthesis in end tissue cells such as bone and muscle.
3. Varicoceles are
   (a) Common and are always associated with hypogonadism.
   (b) Even more uncommon in the infertile/subfertile male than the general population.
   (c) **Extremely common, but they do not cause infertility in most affected men.**
   (d) Most commonly unilateral and found on the right side.
4. Which of the following associations between age and testosterone are true?
   (a) **Both total and free testosterone decline as men age, but the decline in free testosterone is more closely related to symptoms of hypogonadism.**
   (b) Circulating levels of LH decline as men age, leading to decreased production of testosterone within the testicle.
   (c) Atrophy of the hypothalamus associated with age results in a decrease of all pituitary hormones and their downstream hormonal effects.
   (d) Age is associated with decrease in sex hormone–binding globulin production, resulting in decreased levels of circulating bioavailable testosterone.

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Varicocele and Orchialgia

14

Benjamin J. McCormick and R. Matthew Coward

Key Points

- Varicoceles are present in 15% of the male population, and 10% of men with varicoceles experience pain.
- Varicocele-related orchialgia is typically restricted to the scrotum, described as dull, dragging, or heavy, and is worse with prolonged standing.
- NSAIDs, scrotal support, physical activity limitations, and a 6-month-long period of observation is the mainstay initial therapy for symptomatic varicoceles.
- The microsurgical subinguinal approach is the gold standard in surgical repair of varicoceles, with good results and very small rates of complications.
- Up to 90% of men may experience pain relief after varicocele repair.

Introduction

A varicocele is an abnormal dilation and tortuosity of the pampiniform plexus of veins in the spermatic cord [1]. Varicoceles are a common diagnosis that are present in approximately one out of six men of the world's male population [2, 3]. Recently, a large cross-sectional study of over 7000 young men in Europe revealed a prevalence of 15.7% in this population [3]. Varicoceles most commonly affect thin Caucasian men, who typically begin to show signs or symptoms during puberty. Most men with varicoceles are asymptomatic, diagnosed incidentally on routine physical examination or imaging studies. Despite a large asymptomatic population, however, varicoceles are the most common correctable cause of male factor infertility. The prevalence of varicocele increases to 19–41% in men with primary infertility and 45–81% in those with secondary infertility [4, 5]. Additionally, up to 10% of men with varicoceles may experience chronic scrotal pain, also known as orchialgia [6]. Though it is difficult to calculate the number of patient visits related to varicoceles specifically, chronic scrotal pain in general accounts for an estimated 100,000 patient visits per year [7].

Chronic orchialgia is defined as a constant or intermittent scrotal pain, lasting greater than 3 months, and causing significant interference with daily activities. Chronic orchialgia is a difficult diagnosis for the patient and provider alike. For

B. J. McCormick · R. M. Coward (✉)
Department of Urology, University of North Carolina School of Medicine, Chapel Hill, NC, USA

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the patient, chronic orchialgia can be quite severe and detract significantly from his quality of life. For the provider, this notoriously vexing complaint is difficult to treat due to the sheer number of possible etiologies, both urologic and nonurologic in nature. The evaluation of any patient with scrotal pain should include a thorough history and physical examination, in addition to a fertility assessment and imaging studies, if indicated. Pain related to varicoceles is a diagnosis of exclusion, given the high prevalence of varicoceles, their often asymptomatic nature, and the complexity of neural innervation and number of structures contained within the thin-walled scrotum. Therefore, a systematic workup must first be performed to rule out other causes of chronic orchialgia.

The etiology of chronic orchialgia secondary to varicoceles is not well understood. One theory postulates that the dilated veins of the pampiniform plexus compress nearby neural fibers, thereby causing pain [1, 8]. Others theorize that scrotal temperature, oxidative stress, and tissue ischemia may also play a fundamental role. In patients who do not respond to conservative measures, varicocele repair is indicated and provides an effective treatment for pain in appropriately selected patients. In this chapter, we will discuss the presentation of chronic orchialgia related to varicoceles, its management, and outcomes of surgical intervention.

Initial Presentation and Office Evaluation

Patients presenting with chronic orchialgia should undergo a thorough evaluation, even if a varicocele is readily apparent or has previously been implicated as the etiology by a radiologist or other clinician. In addition to a thorough history and physical examination, numerous adjunctive tests are available for use in carefully selected patients. A fertility evaluation is indicated given the strong association with varicoceles and male-factor infertility. Finally, while imaging studies are not routinely required, they can be useful in ruling out other diagnoses or in the setting of a difficult examination.

History

A thorough history should always be obtained, and, in patients presenting with chronic orchialgia, the history often contains the key to unlocking the diagnosis. The history should include the time of onset, severity, location, radiation, and quality and frequency of the pain. A history of an inciting event, such as a sports or work-related injury, speaks more to trauma, hernia, or musculoskeletal etiologies. Severe, acute scrotal pain is almost never the result of a varicocele, which often does not have an inciting event, has been present for years, and results in a low-grade dull ache. Additionally, exacerbating or alleviating factors should be explored. Sexual activity as well as signs of hypogonadism should be explored, including poor libido, erectile dysfunction, and increased fatigue. A complete sexual and reproductive history should be obtained, including new or recent sex partners, number or prior partners, and history of sexually transmitted infections or high-risk sexual behaviors. Chronic orchialgia in the setting of a recent new sex partner or high-risk sexual behavior is most likely to represent an infection. Bowel and bladder habits should be obtained to evaluate for bladder dysfunction or constipation. Many patients with varicocele-related chronic orchialgia are young and physically active, and should be evaluated for any disability related to their pain. This is especially important in patients in the military, police force, or other physically demanding professions where long-term exercise intolerance is unsustainable. Prior surgeries should also be considered, paying special attention to those performed in the abdomen, groin, or scrotum.

Physical Examination

Physical examination of these patients should be equally thorough, as physical examination is the gold standard for the diagnosis of varicoceles [9]. Examination should be performed with the patient in both the standing and supine positions, preferably in a warm room to deter contraction of the dartos and cremasteric muscle fibers. Patients should be

asked to undergo a Valsalva maneuver during the exam, thereby increasing the abdominal pressure and retrograde flow into the pampiniform plexus. While very gently grasping the bilateral cords, these authors prefer to ask the patient to "flex your abdominal muscles" rather than the more common "turn your head and cough" Valsalva maneuver, to perform a better examination of the spermatic cord.

Quantifying the severity of varicoceles is made possible by the simple Dubin and Amelar classification system [10, 11]. This system is based on the ability to identify a varicocele by visual inspection or palpation, with or without the patient performing the Valsalva maneuver. Subclinical varicoceles are not visible or palpable, even with Valsalva, and are diagnosed solely by imaging studies. As per the Dubin and Amelar classification system, grade I varicoceles are only palpable with Valsalva, grade II are palpable without Valsalva, and grade III, the most severe, are clearly visible through the scrotal skin even without Valsalva. These authors prefer to add additional granularity to the grading scale by including I+, II+, and III+ when the varicocele is felt to be in between grades, or, in the case of III+, massively enlarged.

In addition to evaluating for varicoceles, the entirety of the scrotum, groin, and penis should be evaluated for other pathologies. Given that varicoceles can cause testicular atrophy, particular attention should be paid to the testicles, palpating for size, texture, and symmetry. Many cases of chronic orchialgia result from the groin, such as inguinal hernias and groin muscle strains. A thorough examination of the groin, pelvic floor, and inguinal canal is imperative. Testicular pain from infection or inflammation is commonly centered at the epididymis. Gentle palpation of the complete epididymis at the head, body, and tail is necessary to determine the presence of epididymitis and to distinguish scrotal pain between the spermatic cord and the epididymis.

Ruling Out Other Causes of Scrotal Pain

As chronic scrotal pain related to varicocele is a diagnosis of exclusion, evaluation of patients with chronic orchialgia should focus on ruling out other potential causes. Other common causes of chronic orchialgia include spermatoceles, hydroceles, trauma, intra- or paratesticular neoplasms, inguinal hernias, posthernia repair nerve entrapment, and infection/inflammation including prostatitis, orchitis, and epididymitis. All of these causes must be reasonably ruled out prior to attributing chronic orchialgia to a varicocele. As such, careful examination of the entirety of the genitalia and groin is imperative during workup of patients with orchialgia, even when a varicocele is immediately apparent. Beyond a thorough history and physical examination, other diagnostic measures can be considered, depending on the clinical picture. For example, in those with concern for infection, urinalysis and urine culture may be obtained, in addition to testing for sexually transmitted diseases.

In patients with atypical or idiopathic orchialgia, or those with prior inguinal surgery (e.g., inguinal hernia repair, orchiopexy), a spermatic cord block can be a diagnostic and therapeutic treatment option in the properly selected patient. A spermatic cord block is an office procedure in which 10–20 cc of 0.25–0.5% bupivacaine is injected percutaneously into the spermatic cord. The injection is made approximately 1 cm medial and inferior to the pubic tubercle. In patients who experience at least a 50% reduction in pain with a local spermatic cord block, surgical cord denervation may provide sustained relief. Conversely, patients with <50% reduction in pain after spermatic cord block with coexisting varicocele may still benefit from varicocele repair, but may also have unrecognized pathologies including central sensitization, coexisting pudendal pathway, or even malingering, which should be ruled out within reason prior to considering varicocele repair [12, 13].

Fertility Evaluation

Given the strong association with male-factor infertility, patients presenting with a varicocele, regardless of symptoms, should be questioned thoroughly regarding their sexual and fertility

history, even when infertility is not the primary complaint. In addition to the impact of varicocele on spermatogenesis, Leydig cell function can be negatively affected. Poor Leydig cell function can lead to subnormal testosterone levels, and the patient should be evaluated for signs and symptoms of hypogonadism, such as decreased libido, erectile dysfunction, and fatigue.

As varicoceles can cause testicular atrophy, testicular volume should be evaluated. The evaluation should be performed both in the context of absolute terms, and in comparison to the contralateral testis. Orchiometers with standard and graduated volumes can be useful in estimating testicular size. Testicular atrophy, or a testis significantly smaller than the contralateral testis, may experience decreased testosterone production and spermatogenesis. Patients with signs of testicular atrophy should be further evaluated for signs and symptoms of hypogonadism, and offered a fertility evaluation if pursuing fertility is desired.

In addition to a history and physical exam, a laboratory evaluation to further evaluate fertility is an important consideration for a patient presenting with scrotal or testicular symptoms such as those from a varicocele. The minimal reproductive endocrine evaluation would include morning levels of total testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Finally, and perhaps most importantly, a properly collected and analyzed semen analysis is the gold standard examination for male fertility and gonadal function.

Varicocele-Related Orchialgia

Pain experienced by those with symptomatic varicoceles is almost always chronic in nature. Patients with pain lasting for less than 3 months should be carefully evaluated for other causes. Additionally, pain related to varicoceles is most often described as dull, dragging, or heavy. Only rarely is pain described as sharp or stabbing, which could point toward other etiologies. Most commonly, the pain is isolated to the ipsilateral testicle, scrotum, and/or groin, rather than radiating to the inner thigh or contralateral side. Given that most varicoceles develop during puberty, older men presenting with new-onset scrotal pain should have a thorough evaluation, even if a varicocele is the only identifiable etiology; in this population, new-onset scrotal pain is rarely caused by a varicocele.

Imaging Studies

The role of adjunctive diagnostic imaging is not routinely indicated for thin patients with unilateral left-sided varicoceles, except for when pain is the presenting complaint. Imaging can also be useful in the setting of difficult or indeterminate physical examinations, such as in the setting of obesity or prior surgery. In these cases, scrotal ultrasonography has a very high sensitivity (97%) and specificity (94%) and can aid in diagnosis. Other diagnostic imaging studies, such a radionuclide scanning, thermography, and spermatic venography, should only be considered in the case of recurrent varicocele.

The presence of a varicocele by ultrasonography is determined by dilation of spermatic veins with demonstration of reversal of flow with color Doppler. Neither dilation of spermatic veins nor reversal of flow alone is sufficient for the diagnosis of varicocele, as patients having previously undergone repair can have persistently dilated veins without flow reversal. A commonly used cutoff for dilated spermatic veins is 3 mm in diameter, as this size appears to correlate with the ability to palpate the varicocele on physical exam. However, operator variability and a lack of standardized criteria make definitive correlation between vein diameter and palpable varicoceles difficult. Likewise, no correlation between vein diameter and likelihood or severity of pain has been observed.

In summary, we define varicocele related-orchialgia as scrotal pain lasting greater than 6 months in patients with a varicocele, and in which the pain cannot be attributed to another cause despite a thorough investigation. The history and physical examination are the primary means of diagnosis. The pain is most commonly

experienced by young men, is almost always dull, heavy, or dragging in character, and is isolated to the ipsilateral scrotum. No routine imaging is indicated for unilateral left varicoceles, but is indicated in nonreducibile or unilateral right varicoceles as this may indicate occult retroperitoneal pathology. Further studies (e.g., urine culture, CT/MR imaging, semen analysis) can be ordered as indicated based on the history and physical exam. A spermatic cord block can be a useful diagnostic and therapeutic tool in patients with chronic idiopathic scrotal pain, or pain that is not completely consistent with varicocele-related orchialgia.

Conservative Management

Initial management of chronic orchialgia presumed to be related to a varicocele should always include conservative measures and a generous period of observation. Conservative treatment includes the use of nonsteroidal anti-inflammatory medications (NSAIDs), scrotal support, and limitation of strenuous physical activity. These authors prefer daily use of prescription-strength NSAIDs, as opposed to recommending over-the-counter formulations, which are more often used by patients on an as-needed basis only. Scrotal support can be accomplished with an athletic supporter or compression shorts, although we prefer to recommend underwear with a specific medical indication of scrotal support such as UFM Underwear™. After implementing these measures, 4–15% of men can experience significant symptomatic relief [14, 15]. Though there is no consensus regarding the amount of time for conservative measures, we recommend waiting at least 6 months prior to considering surgical options in order to allow for improvement or resolution of the pain or for other etiologies to present. Some patients will not be able to limit their activity due to professional demands (e.g., military, athletes, police). In this case, if scrotal support and NSAIDs are not sufficient, informed and shared decision making should be offered prior to moving forward with procedural interventions.

Procedural Management

The goal of procedural intervention for repair of varicoceles is ligation of the internal spermatic and cremasteric vein(s) to prevent retrograde flow into the pampiniform plexus, thereby encouraging testicular venous drainage via the vasal veins, which exit the spermatic cord with the vas deferens and ultimately drain into the pelvis via the internal iliac vein. There are several methods by which ligation of the internal spermatic vein can be accomplished, including endovascular, laparoscopic, and open surgery. Approaches for open surgery include high retroperitoneal (Palomo), inguinal (Ivanissevich), and subinguinal; each approach has unique benefits and drawbacks. Open surgery can be further augmented with the use of Loupe magnification and the surgical microscope. Outcomes of open repair of varicoceles for pain are good, regardless of approach, with over 90% improvement or resolution in pain. With a short recovery time and the lowest rate of complications, open microsurgical subinguinal repair is the gold standard. The only drawback to the subinguinal microsurgical approach is the microsurgical training and equipment required.

Indications for Repair

Historically, guidelines have reserved varicocele repair only for men with male-factor infertility combined with subnormal semen parameters. However, over the past several years, guidelines have evolved and now recommend varicocele repair in patients with varicocele-related chronic orchialgia. Specifically, in 2014, the American Society for Reproductive Medicine (ASRM) along with its affiliate Society for Male Reproduction and Urology (SMRU) published guidelines recommending varicocele repair for persistent varicocele-related scrotal pain that does not improve with conservative management, regardless of fertility status [16]. These guidelines reflect an expanding body of evidence that supports varicocele repair in patients with varicocele-related chronic scrotal pain.

Predictors of Success

While outcomes of varicocele repair for pain are overall quite good, specific characteristics of varicoceles may predict a higher likelihood of pain relief with repair. One such characteristic is the duration of pain. In one study of 284 patients, 7% of patients had persistent orchialgia after varicocele repair; of patients with persistent pain, 89% had pain that began less than 3 months prior to surgery [17–19]. This finding emphasizes the need for a genuine attempt at conservative therapy for at least 3 months; indeed, these authors prefer conservative measures for 6 months before considering procedural intervention.

Besides the chronicity of pain, some suggest that patients with higher-grade varicoceles are more likely to experience pain relief after surgery [14]. Other studies suggest that the character of the pain itself is predictive of successful repair. Pain that is restricted to the scrotum and described as dull, dragging, or heavy is more consistent with varicocele-related pain and may be more likely to improve after surgery. Patients with higher preoperative pain scores may also see more benefit from varicocele repair. Pain that radiates to the groin or inner thigh, or pain that is described as sharp or stabbing, may still be related to the varicocele, but may also be less likely to improve after surgery. Finally, if more than 7 veins are ligated during subinguinal varicocele repair (described in the following section), they may also positively impact pain relief after surgery [15]. Other factors such as body mass index and pain severity have not shown reliable correlation with pain relief after surgery.

Adjuncts to Surgical Repair

Loupe magnification, microvascular Doppler ultrasound, and the operating microscope are useful adjuncts to open surgical repair, which aid in identification of the small and delicate structures in the spermatic cord. Correct identification of the spermatic veins, arteries, and lymphatic channels is essential to a successful varicocele repair and avoiding complications. When available, the operating microscope should be used. The operating microscope is superior to Loupe magnification for the identification of internal spermatic veins, arteries, and lymphatics [20]. In addition, the microvascular Doppler is a useful tool for distinguishing between internal spermatic arteries and veins. Patients undergoing Doppler ultrasound-assisted repair have an increased number of spermatic arteries identified and spared, and ultimately have improved semen parameters postoperatively [21]. Loupes, operating microscopes, and microvascular Doppler, all serve as useful tools in the urologist's armamentarium, and should be used when possible.

Surgical Repair of Varicoceles

Historically, the first commonly performed surgical procedure for the repair of varicocele was via a scrotal approach. Given the intimate relationship between the pampiniform plexus and the testicular arteries at this level, however, injury to the testicular artery was common, and this approach was abandoned in favor of the three contemporary surgical techniques: retroperitoneal, laparoscopic, and microsurgical (inguinal or subinguinal).

In the open retroperitoneal approach, a 3–5-cm horizontal incision is made inferior and medial to the ipsilateral anterior superior iliac spine and extended medially over the internal inguinal ring. The external and internal oblique muscles are divided, and the transversalis fascia is opened. Next, the retroperitoneum is reflected medially. The gonadal vein is identified lateral to the ureter and ligated. The primary advantage of retroperitoneal repair is the simplicity that this approach affords. The gonadal vein at this level of ligation is large and either solitary or very few in number, making identification easier. The drawback of retroperitoneal repair is the high incidence of recurrence due to collateral venous drainage between the high ligation point and the testicle. Given this higher recurrence rate, the retroperitoneal approach is now rarely used in contemporary surgical practice and is mentioned here for completeness.

A laparoscopic approach can also be used in the repair of varicoceles. The laparoscopic approach carries all of the benefits of open retroperitoneal repair, as well as several others, which makes it preferable to open retroperitoneal repair, including increased magnification and decreased pain and recovery time. Additionally, the laparoscopic technique is safe even after prior inguinal surgery. In this technique, insufflation of the abdomen is achieved, and ligation of the spermatic vein(s) is performed after dissection of the spermatic cord through the posterior peritoneum ~5 cm proximal to its entry into the internal inguinal ring, well away from vas deferens. Doppler ultrasound can also be used in this technique, helping to differentiate veins from arteries. The disadvantages of a laparoscopic approach mirror those of the open retroperitoneal technique, including a higher incidence of recurrence relative to inguinal and subinguinal techniques, described in the following section. Laparoscopic varicocele repair remains common practice, however, in the pediatric population. In small children, the spermatic vessels are often too small to reliably identify, even with surgical microscopy and microvascular Doppler ultrasound. In this population, this method offers a minimally invasive technique that portends good results. For adults, however, the authors recommend against this technique in favor of the subinguinal approach.

The most frequently utilized surgical approaches are the inguinal and subinguinal approaches. Visualization and safety are greatly improved with each approach using the operating microscope. The inguinal approach is performed via an incision superior to the external inguinal ring. The fascia of the external oblique muscle is incised, this exposes the spermatic cord and it is isolated and the internal spermatic veins are ligated. At this level, the veins are larger than those seen with the subinguinal approach, and as such, this approach may be performed with loupes if an operating microscope is not available. The primary disadvantage to this approach is the higher rate of recurrence due to the external spermatic, or cremasteric, veins not available at this level as they enter the cord when it exits the external ring. Additionally, the need to incise the external oblique fascia causes more postoperative pain and longer convalescence.

The microsurgical subinguinal approach to varicocele repair is the gold standard surgical approach to varicocele repair. With both the inguinal as well as subinguinal approaches, identification of the internal and external spermatic arteries is greatly enhanced with the addition of the microvascular Doppler ultrasound. It is particularly useful with the subinguinal approach, since the arteries may be even smaller in diameter and the number of veins is greater. This approach involves a 2-cm incision inferior to the external inguinal ring and does not require incision of the external oblique fascia. Not requiring a musculofascial incision reduces postoperative pain and recovery, which is a primary advantage of this approach. It has the lowest rate of complications, and additionally, this approach has the lowest rate of recurrence due to the ability to ligate the external spermatic, or cremasteric, veins at this level [22].

Microscopic Denervation of the Spermatic Cord

Denervation of the spermatic cord refers to a surgical procedure in which all the nervous tissue of the spermatic cord, including the ilioinguinal nerve, spermatic branches of the genitofemoral nerve, and autonomic fibers in the cord, are divided. We perform this procedure primarily in patients with chronic idiopathic orchialgia, which has been exhaustively evaluated and does not improve with conservative therapy. We may also perform this procedure as an adjunct to varicocele repair in men who are not interested in future fertility, or have atypical pain symptoms not typically attributed to varicocele-related orchialgia (e.g., pain that is sharp or radiating to groin/thigh, older men).

Cord denervation is performed using the same approach as the microsurgical subinguinal varicocele repair, described earlier. In spermatic cord denervation, however, all arterial and identifiable lymphatic structures are iso-

lated and spared, while the nervous tissue, cremasteric muscle fibers, and testicular veins are ligated. In men with no desire for future fertility, the vas deferens is also divided, removing its sympathetic innervation. Ligation of the vas deferens at this level may be helpful in men who have previously undergone vasectomy to relieve any component of pain related to post-vasectomy orchialgia. At the conclusion of cord denervation, all that remains of the spermatic cord is the testicular artery, the deferential artery, and what few lymphatic vessels are identified and spared.

Outcomes of Varicocele Repair for Pain

As demonstrated in Table 14.1, there is a distinct lack of prospective randomized control trials examining the outcomes of varicocele repair on pain. The vast majority of data presented in this chapter is based on retrospective studies, and as such brings with it some inherent weaknesses. Nevertheless, from the available literature, we believe that surgical repair of varicoceles performed for chronic orchialgia results in improvement or resolution of pain in 83–100% of patients, with the average estimated at about 92% [23, 24]. The largest contemporary series describes 237 men undergoing surgical repair of varicocele for pain. In this study, 86% of patients experienced complete resolution of pain after subinguinal microscopic varicocele repair and 92% experienced significant improvement [19]. Another study examining patients undergoing retroperitoneal repair of varicocele for pain demonstrated pain relief in 90% of patients [25, 26]. Regardless of approach, surgical intervention is effective in the vast majority of patients with varicocele-related orchialgia. As discussed earlier, we prefer the subinguinal microsurgical approach due to its high success and low rates of complications.

While most patients undergoing varicocele repair for pain endorse significant pain relief, the minority of patients who experience persistence or recurrence of testicular pain after varicocele repair pose a unique challenge. Treatment failure may represent incorrect diagnosis, underlying idiopathic orchialgia, unsuccessful repair, or a surgical complication. For all patients with treatment failure, the first step is re-evaluation of the initial diagnosis, ensuring an appropriate evaluation was performed. In patients with a persistent or recurrent varicocele after attempted nonmicrosurgical repair, there has been published success with subsequent microsurgical subinguinal repair, with up to 90% of patients experiencing an improvement in pain [27]. In patients with failed inguinal microsurgical varicocele repair, we recommend percutaneous embolization of the gonadal vein, given the difficulty of reoperation in the groin. Finally, for patients with resolution of their varicocele, but with persistent pain, we offer microscopic subinguinal cord denervation (for prior retroperitoneal or high inguinal repair) or orchiectomy (for prior subinguinal repair, and only after exhaustive counseling). As in every case, careful patient selection and managing expectations are vital.

Percutaneous Embolization

Percutaneous endovascular embolization of the internal spermatic vein is the primary alternative to surgical varicocele repair. Endovascular embolization uses metal coils or other thrombosing agents (e.g., alcohol) to effectively occlude the gonadal vein. The technical success of endovascular is variable, and depends largely on the experience of the interventional radiologist. One series demonstrated failed access to the gonadal vein in 19% of cases [28, 29]. When access is gained successfully, however, efficacy appears to be similar to that of high ligation surgical techniques (laparoscopic, open retroperitoneal), with recurrence rates up to 13% [30]. As such, endoscopic repair is now more commonly reserved for patients with recurrent varicocele after prior subinguinal repair (as attempted repeat repair for failed high ligation or inguinal approach may still be approached subinguinally). In this setting, radiographically defining the venous anatomy and direction of blood flow is useful and effective [31].

Table 14.1 Surgical approach to varicocelectomy for pain

Study	Year	Study design	Control group	Patients (*n*)	Mean patient age (*y*)	Mean duration of pain (months)	Approach	Magnification	Doppler ultrasound	Complete resolution of pain	Improvement in pain	Persistent pain
Peterson [6]	1998	Retrospective	No	35	25.7	17.8	Inguinal, subinguinal, retroperitoneal, laparoscopic	None	No	86% (*n* = 30)	89% (*n* = 31)	11% (*n* = 4)
Yaman [14]	2000	Retrospective	No	82	Not described	Not described	Subinguinal	Microscope	No	88% (*n* = 72)	94% (*n* = 77)	6% (*n* = 5)
Maghraby [26]	2002	Retrospective	No	58	21.5	5.2	Laparoscopic	N/A	No	84% (*n* = 49)	94% (*n* = 55)	6% (*n* = 3)
Tung [32]	2004	Retrospective	No	27	28.3	Not described	Subinguinal	None	No	90% (*n* = 28)	100% (*n* = 31)	0% (*n* = 0)
Chawla [27]	2005	Retrospective	No	11	Not described	Not described	Subinguinal	Microscope	No	54% (*n* = 6)	91% (*n* = 10)	9% (*n* = 1)
Karademir [24]	2005	Prospective	No	121	21.1	17.3	Inguinal, subinguinal	None	No	61% (*n* = 74)	84% (*n* = 101)	16% (*n* = 19)
Altunoluk [19]	2010	Retrospective	No	237	23.7	11.2	Subinguinal	Microscope	No	86% (*n* = 203)	92% (*n* = 218)	8% (=19)
Parekattil [33]	2011	Retrospective	No	45	Not described	Not described	Robotic	Robotic microscope	Yes	92% (*n* = 42)	–	–
Abd Ellatif [18]	2012	Retrospective	No	130	25.7	17.8	Inguinal, subinguinal	None	No	84% (*n* = 109)	89% (*n* = 116)	11% (*n* = 14)
Kim [23]	2012	Retrospective	No	81	22.4	22.8	Subinguinal	Microscope	No	72% (*n* = 58)	91% (*n* = 74)	9% (*n* = 7)
Kachrilas [25]	2014	Retrospective	No	48	38.2	Not described	Laparoscopic	N/A	No	88% (*n* = 42)	98% (*n* = 47)	2% (*n* = 48)

Conclusion

Varicoceles occur in about 15% of men, and are the most common correctable cause of male-factor infertility. Additionally, chronic orchialgia can occur in approximately 10% of the affected population. Varicocele-related orchialgia is a diagnosis of exclusion, and a thorough history (including complete sexual and reproductive history) must be obtained when evaluating patients with varicoceles. Physical examination is the primary method of diagnosis, and imaging studies should only be performed to rule out other sources of pain. Further investigation must be performed, however, in patients with an isolated right-sided varicocele, as this may predict retroperitoneal pathology. Conservative management is effective in many cases and should include NSAIDs, scrotal support, and activity restrictions for 6 months. When pain persists despite conservative measures, surgical intervention is indicated. The microsurgical subinguinal varicocele repair is the gold standard for treatment of varicocele-related chronic orchialgia and, based on limited and retrospective data, appears effective at relieving or resolving pain in up to 90% of cases. Using this technique with the assistance of Doppler ultrasonography, complications such as testicular artery injury, hydrocele formation, and recurrence of varicocele are negligible.

Review Criteria

An extensive search of studies examining the relationship between varicocele and pain was performed using search engines such as ScienceDirect, OVID, Google Scholar, PubMed, and MEDLINE. The start and end dates for these searches were January 1995 and June 2018, respectively. Articles published in languages other than English were also considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included.

Multiple Choice Questions and Answers

1. A 60-year-old male presents with 3 months of right groin pain and is found to have a right grade II varicocele. He states that the pain is dull, isolated to the right hemiscrotum, and is worse at the end of the day. The next best step is.
   (a) Prescribe nonsteroidal anti-inflammatory medications, scrotal support, and physical active restricts with follow-up in 6 months.
   (b) Scrotal ultrasound.
   (c) Bilateral endovascular internal spermatic vein ablation.
   (d) **CT abdomen and pelvis with contrast.**
2. The most likely neurologic deficit to be encountered after laparoscopic varicocele repair is.
   (a) Paresthesia of the penile shaft.
   (b) **Numbness of the ipsilateral inner thigh.**
   (c) Absent ipsilateral cremasteric reflex.
   (d) Numbness of the posterior scrotum.
3. Percutaneous embolization for repair of varicoceles is most useful in which setting?
   (a) Patients with unilateral right-sided varicoceles.
   (b) Obese patients with prior abdominal surgery.
   (c) **Patients with recurrent postoperative varicoceles.**
   (d) Patients with grade II or grade III varicoceles.
4. A 22-year-old obese male presents with 6 months of dull left scrotal pain that is worse with prolonged periods of standing. The physical exam is equivocal due to his body habitus. He was previously told he had a varicocele on the left, but he has never before experienced symptoms. The patient would like to avoid surgery if possible. The next best step is.
   (a) **Scrotal ultrasound.**
   (b) Conservative management with NSAIDs, scrotal support, and physical activity restrictions.
   (c) Percutaneous selective internal spermatic vein embolization.
   (d) Microscopic subinguinal varicocele repair.

5. A 25-year-old Marine presents with a several-year-long history of isolated left scrotal pain that interferes with his duties. He states the pain is dull, heavy, and dragging, and is worse with physical activity. Scrotal ultrasound at his referring physician's office confirms a large left varicocele, and is otherwise normal. He is found to have a grade III left-sided varicocele, which he states has been present since age 13. He states he is deploying overseas in 2 months and is in dire need of relief.
   (a) Conservative management with NSAIDs, scrotal support, and physical activity restrictions. Return to clinic in 6 months.
   (b) **Left subinguinal microsurgical varicocele repair.**
   (c) Scrotal ultrasound.
   (d) Percutaneous internal spermatic vein embolization.

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Part III

Varicocele Therapy

15 Medical Therapy in Varicocele-Related Infertility

Gian Maria Busetto, Francesco Del Giudice, and Ettore De Berardinis

Key Points

- Varicocele is considered as one of the initiators of oxidative stress, which causes an altered balance between production and clearance of ROS. This mechanism is the cause of spermatogenic defect.
- Excessive ROS are detrimental to cell function and survival, but small quantities of ROS are required to maintain a correct sperm maturation and function.
- Medical treatments available for varicocele are antioxidants, hormonal agents, and Chinese medicine.
- Medical therapy for varicocele requires more evidence and more DBPC studies, evaluating correct regimen and considering the effect of standardized doses in large trials.
- Varicocelectomy is currently the treatment of choice for OAT, secondary to varicocele. Drugs alone cannot be suggested, while there is a room for medical therapy after surgery to possibly hasten the restoration of seminal parameters.

Introduction

Male factor can be considered the cause of infertility in up to 60% of couples [1]. The etiology of male infertility is known in approximately 70% of the cases, while the remaining 30% could be considered as idiopathic infertility (normal findings on physical examination and endocrine, genetic, and biochemical laboratory testing). Varicocele, among the known causes of male infertility, is the most frequent and is reported in up to 15% of infertile men [2–3].

Even if, to date, many conditions related to infertility have been understood, there are still significant number of patients who are diagnosed with idiopathic oligoasthenoteratozoospermia (OAT). For this reason, all available medical and surgical treatments are performed to correct any possible curable cause [4]. In recent years, efforts against male infertility are decreasing, particularly after the emerging role of assisted reproductive

G. M. Busetto (✉) · F. Del Giudice · E. De Berardinis
Department of Urology, Sapienza Rome University, Policlinico Umberto I, Rome, Italy
e-mail: gianmaria.busetto@uniroma1.it

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technology (ART), following the introduction of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Notably, the progressive decline in male fertility worldwide is a concern. The latest findings reveal that, between 1973 and 2011, the concentration of sperm in the ejaculate of men in western countries has fallen by an average of 1.4% per year, leading to an overall drop of over 52% [5]. It is important to note that ART is not able to solve all infertility problems and the overzealous use of ART is not without ethical implications. Improving sperm quality and male fertility potential is important to achieve better reproductive outcomes in both natural conception and assisted reproduction.

Before understanding the role of medical treatment in male infertility, it is important to understand the relationship between sperm quality and fertility potential. Even if a strong relationship has been postulated, this relation is still far from being confirmed. Many trials reported that sperm characteristics are related to fertility, and this is more evident when low sperm parameters are recorded [6, 7]. Following these results, it is important to reverse any known cause of infertility to obtain a better sperm quality and to avoid more invasive and expensive procedures such as ART.

In spite of varicocele being one of the most important causes of male infertility, it is still a highly debated issue. In fact, on one side, not all men suffering from varicocele have impaired semen parameters; on the other, not all men undergoing varicocele treatment will have an improvement in fertility [8–9]. Literature is reporting many trials on this topic, but mechanisms explaining pathophysiology of infertility related to varicocele are still lacking.

Varicocele Causing Male Infertility

To date, there are four main theories explaining this issue:

1. The scrotal and testicular hyperthermia could be the cause of altered spermatogenesis because enzymes responsible for DNA synthesis are temperature-sensitive. A better spermatogenesis takes place at a temperature lower than our body, and even enzymes work optimally at a lower temperature. Varicocele, with an increased blood flow, is responsible for an increase in temperature [10, 11].
2. Spermatogenesis can be altered because of an increase in testis blood flow, changing paracrine communication between Leydig cells and Sertoli cells with peritubular and myoid cells. Interstitial fluid composition is altered together with transmembrane transport of substrate [12, 13]. Testosterone production, even if not directly correlated with spermatogenesis, is altered because of Leydig cells' decreased production under varicocele stress. In this toxic environment, even conversion from cholesterol precursors to testosterone is more difficult.
3. Stasis of blood in the pampiniform plexus, and reflux of renal/adrenal metabolites in the testis may negatively act on spermatogenesis. In varicocele patients, blood reflux has been demonstrated, but related hypoxia has not been reported. Even renal and/or adrenal metabolites can cause a damage in the testis, but the detrimental effect of varicocele on testicular function remains the same after adrenalectomy in rat model [14–16].
4. Varicocele is associated with a wide range of hormonal abnormalities causing damage during sperm maturation. Testosterone (T) levels, important for spermatogenesis, could be affected by varicocele, and testosterone production is under the control of luteinizing hormone (LH). An increase in LH could alter the hypothalamic-pituitary-gonadal axis, together with gonadotropin-releasing hormone (GnRH). Even follicle-stimulating hormone (FSH) and inhibin, acting on Sertoli cells, are often altered in varicocele patients and could create certain problems in sperm production. The paracrine regulation of the testis involves Fas, a transmembrane receptor protein expressed by germ cells, and several epidermal and vascular endothelial growth factors. Finally, free radicals increased with varicocele, acting on the testis, could be included in the paracrine regulation [17–22].

Oxidative Stress and Spermatogenesis Defect

All these mechanisms are considered to be the initiators of an oxidative stress (OS) process causing spermatogenesis defect. Sperm require oxygen as an essential substance for their maturation and function. Reactive oxygen species (ROS), present as free radicals, are derived from oxygen and are necessary for maintain normal cell function. Conversely, excessive ROS are also detrimental to cell function and survival. Common forms of ROS are classified as radical (hydroxyl ion, superoxide, nitric oxide) and non-radical (hydrogen peroxide, lipid peroxide, singlet oxygen, ozone), and reactive nitrogen species are considered as ROS subclass (nitrous oxide, peroxynitrite, nitroxyl ion) [23].

Small quantities of ROS are required to maintain a correct sperm function, and antioxidants help keeping the correct balance by avoiding an excessive increase of ROS and their harmful effect. It is when there is an uncontrolled change of the equilibrium between ROS production and clearance, in favor of accumulation, that oxidative stress takes place and sperm damages are reported [8]. For a good spermatogenesis, it is fundamental to maintain the correct amount of ROS to avoid OS. In fact, for a normal sperm cell function, including chromatin compaction in maturing spermatozoa during epididymal transit, a delicate redox balance between reduction and oxidation is required [24]. Furthermore, acrosome reaction, hyperactivation, motility, and capacitation require low levels of ROS. Spermatozoa have very high energy requirements because sperm functions such as capacitation and motility are all highly energy-dependent. Mitochondria are responsible for energy metabolism, and their dysfunction can represent a negative effect for semen quality through decrease in energy availability [25]. Spermatozoa plasma membranes and cytoplasm are rich in polyunsaturated fatty acids (PUFA), which are vulnerable to ROS. Elevated ROS exposure leads to membrane damage, instability, and functional alterations, causing cell death [26]. Recent evidence shows an association between high ROS levels and increased mitochondrial DNA (mtDNA) copy number with decreased mtDNA integrity [27]. In general, an oxidative increase may lead to cellular degeneration by apoptosis or necrosis, while a reduction could facilitate cell survival. There are two major sources of ROS: leukocytes and spermatozoa. Gomez et al. reported an interesting correlation between ROS generation and cytoplasmic droplets or excess residual cytoplasm. Semen cytoplasmic extrusion could be altered and immature or defective spermatozoa, with a surplus of residual cytoplasm, are released [28].

For spermatozoa ROS production, two possible sources have been proposed: sperm plasma membrane nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system or mitochondrial NADPH-dependent oxidoreductase [29]. Mitochondria are required for energy metabolism, while a damaged mitochondrion can be a cause of increased oxidative stress. OS recognize several targets that depend not only on ROS quantity but also on time of exposure, temperature, and surrounding environment.

Oxidative Stress and Pathophysiology of Varicocele

Correlation between varicocele and oxidative stress has been extensively reported. Agarwal et al., in a meta-analysis, reported a statistically significant difference in oxidative stress parameters between patients with varicocele and controls [30].

Several studies have shown a direct, temperature-dependent relationship between heat exposure and ROS generation. Heat stress damages have been reported in cultures of mouse and rabbit spermatozoa and in several human cell lines [31–32].

Functional levels of NO are able to enhance sperm motility, while excessive levels can result in sperm immobilization and testicular sperm apoptosis [33]. Impaired fertility in patients with varicocele may be explained based on the findings of elevated NO/NOS levels in systematic circulation and spermatic veins [8].

Comparing patients with and without varicocele and including a correlation with varicocele grade, Shirashi et al. found higher NO levels in the intratesticular fluid, higher iNOS expression in testicular biopsy samples of varicocele subjects, and no eNOS or nNOS expression difference between subjects [34]. These results report that the upregulation of iNOS is the main source of NO generation in varicocele, causing cellular injury and apoptosis. Venous stasis of varicocele and related hypoxia could be overcome with a vasodilator effect by endothelial cells' NO increase, and this can be considered as an another mechanism of NO production [35]. Free radicals generated inside the mitochondria can react with NO producing active metabolites, and high levels of NO can even inhibit ATP production acting on mitochondrial oxygen respiration [36].

Mitochondria under heat stress of varicocele are able to directly produce ROS, and this can be explained with different theories. Mitochondria respiratory chain and electron flux can be disrupted with an "absorption" of electrons from oxygen molecules, which increase ROS production and decrease ATP synthesis [8]. Another mechanism is based on the acceleration of energy expenditures by cellular metabolism again with an increase in ROS production. Last hypothesis, based as well on increase in cell metabolism, reports that hypoxia is responsible for ROS production because there is an increase in blood flow [37].

Xanthine dehydrogenase/oxidase catalyzes conversion of xanthine to hypoxanthine and uric acid. Under hypoxia or heat increase, this enzyme can be converted to its oxidase form by reversible sulfhydryl oxidation or irreversible proteolytic modification. Working in this altered condition results in concomitant ROS production increase and ATP production decrease favoring ADP accumulation [38]. Even an excessive presence of xanthine oxidase, increasing 8-hydroxy-2′-deoxyguanosine (8-OHdG), can be the cause of different DNA damages. [39].

The membrane protein HO1 (haeme oxygenase 1) has an important role in regulation and cell protection and is induced in response to different stressors. This protein can degrade haeme, forming biliverdin and bilirubin. A significantly higher levels of haeme oxygenase and bilirubin have been reported in varicocele patients, compared to healthy men and this can be considered another cause of testicular damage [40].

Lastly, heat-shock proteins (HSPs) that are activated during heat exposure, hypoxia, or oxidative stress increases. Different damages are reported to cellular protein components, resulting in their denaturation, misfolding and aggregation, and finally cell apoptosis. Inadequate concentrations of HSPs (HSPA2 and HSPA4 most studied) are often reported in patients with varicocele leading to increase in sperm damage [41].

This detrimental effect is partially balanced through an increase in specific antioxidants, such as coenzyme Q10, vitamin C and E, glutathione peroxidase, superoxide dismutase, and catalase [42, 43]. Another strong correlation is reported between ROS and varicocele grade. Due to this, levels of seminal ROS, malondialdehyde, H_2O_2, NO, and 8-OHdG are increased significantly in higher varicocele grades [44]. Despite all these mechanisms, not all patients with varicocele are infertile and many of them still have a good fertility potential. The phenomenon may be explained by the varying ROS concentration and subtypes. Fertility is maintained in some patients with varicocele due to the difference in response to OS which is related to underlying genetic variation [43, 45]. Patients subjected to varicocelectomy are more protected from oxidative stress because of improved sperm quality and increased antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, and vitamin C [46].

Several tests have been developed to quantify OS. For example, malondialdehyde, a byproduct of lipid peroxidation, is commonly used and can be measured with TBARS (thiobarbituric acid reactive substances) assay or chemiluminescence [47].

In the presence of a free electron, oxidative species bind to the cell membrane, which contains the electron-rich polyunsaturated fatty acids (PUFA). This interaction results in the transfer of

an electron from the membrane to the reactive species, bringing to radical formation. The amplification of this reaction through several cycles results in the production of malondialdehyde that can be measured [8].

Even direct ROS evaluation and total antioxidant capacity (TAC) have been implemented. All these methods report a significant difference between the values obtained in infertile men with varicocele and in fertile men or infertile men with idiopathic infertility [48].

Medical Treatment for Varicocele

When considering surgery as an effective option for varicocele treatment, recurrence and complications are the drawbacks. Furthermore, desirable outcomes do not always occur after surgical intervention in patients with varicocele. Therefore, medical therapy represents an alternative and a less-invasive treatment option for varicocele.

Medical treatment is a feasible option and, to date, there are the following therapies:

1. Antioxidant agents that are able to decrease oxidative stress and lower ROS levels. Many elements have been studied and can be used as antioxidant agents. Currently, carnitines, selenium, coenzyme Q10, vitamin C and E, vitamin B12, bioflavonoids, lycopene, kallicrein, cinnoxicam, pentoxyfilline, zinc, folic acid, GSH, and NAC are used.
2. Hormonal agents like gonadotropins, tamoxifene, clomiphene, and menotropin that should restore hormone levels.
3. Chinese medicine with a multitude of antioxidant and anti-inflammatory agents. The most common products are qianjing, guizhi fuling wan, escin, jingling, and green tea.

Table 15.1 Antioxidant therapies

Element	Action	Evidence	References
L-carnitine and acetyl-L-carnitine	Improve energy metabolism and facilitate mitochondrial ATP production.	Effective in patients with OAT with or without varicocele. Improve sperm parameters and pregnancy rate.	[49, 53–59]
Cinnoxicam	Anti-inflammatory action.	Effective in patients with OAT when associated with carnitines. Improves sperm parameters.	[54]
Selenium	Action through glutathione peroxidase enzymes.	Improves sperm parameters in patients with varicocele.	[58, 59]
Vitamin E	Scavenge free radicals and decrease lipid peroxidation.	Increase of antioxidant status in patients with and without varicocele. Improves pregnancy rate.	[60–62]
Vitamin C	Chain breaking action, keeping balance between oxidants and antioxidants.	Increases sperm count and reduces 8-OHdG. Effective in patients with and without varicocele.	[60, 63, 64]
Zinc	Antioxidant role maintaining a correct ROS regulation.	Improves sperm parameters in patients with and without varicocele (when associated with folic acid and pentoxifylline).	[65, 66]
Coenzyme Q10	Protection against peroxidative damages through low-density lipoproteins.	Effective in patients with OAT with or without varicocele. Improves sperm parameters.	[67–69]
GSH and NAC	Action over DNA damage and protection on sperm impairment.	Decrease in ROS levels and increase in sperm parameters.	[70–74]
Kallikrein	Increase testosterone and sperm maturation.	Increase in sperm motility and morphology in patients with varicocele.	[75]
Bioflavonoids	Cell cycle inhibitors. Venous tone increase in varicocele.	Improve sperm parameters in patients with varicocele. Relieve varicocele pain and improve Doppler ultrasound parameters.	[76–80]

Antioxidant Therapy (Table 15.1)

Nonenzymatic antioxidants including vitamins (mainly vitamins A, B, C, E), glutathion, metabolic coenzymes such as pantothenic acid, coenzyme Q10, carnitines (L-carnitine and acetyl-L-carnitine), and micronutrients (zinc, selenium, copper) are often deficient, causing a general decrease in the antioxidant status as well as mitochondrial dysfunction [49–50]. Nutrients such as zinc, folic acid, vitamin B12, L-carnitine, and acetyl-L-carnitine are required for sperm formation and maturation [51, 52].

Several studies demonstrate that these products could positively act on fertility through an improvement of sperm quality and are, therefore, recommended as potentially effective therapy for the treatment of male infertility. A therapeutic strategy would need to use supplements to increase sperm energy metabolism, minimize free radical damage to sperm, and improve the cellular processes connected with the formation and maturation of sperm [49]. Antioxidants are able to protect sperm from ROS damages, decrease DNA fragmentation, reduce cryodamage to spermatozoa, block premature sperm maturation, and improve outcomes of assisted reproductive techniques (ART).

Mostly, antioxidants can be divided into three groups:

1. Dietary antioxidants like vitamin C and E, bioflavonoids, beta-carotenes, and carotenoids.
2. Endogenous antioxidants like glutathione peroxidase and reductase, catalase, SOD, albumin, pyruvate, vitamin E and A, ubiquitol, ascorbate, urate, taurine, and hypotaurine.
3. Metals that bind with certain proteins enhancing their effect (proteins reducing OS) like albumin, ceruloplasmin, trasferrin and ferritin, myoglobin, and metallothionein.

L-carnitine and acetyl-L-carnitine are common and safe for treating male infertility; their action is based on the capacity to improve sperm quality and pregnancy rate in males suffering from asthenoteratozoospermia [53]. Association of L-carnitine and acetyl-L-carnitine has been reported by Cavallini et al. to be effective in the treatment of sperm alterations. Subjects have been divided between varicocele and OAT patients and received placebo or L-carnitine + acetyl-L-carnitine. Sperm concentration, motility, morphology, and pregnancy rate have been assessed, and all of them improved significantly after treatment [54]. Sofimajidpour et al. reported, in their trial, an interesting comparison between surgical and medical treatment with carnitines in patient with grade II varicocele. They concluded that both treatments are effective in treating varicocele and that difference between the two is not statistically significant [55]. There are negative studies which demonstrated lack of improvement in fertility after medical treatment. However, the study population were patients with impaired semen parameters in the absence of varicocele [56–58]. Carnitines are safe products with rare side effects; only sporadic cases of nausea, vomiting, stomach upset, heartburn, diarrhea, and seizures have been described [49, 53, 59].

Cinnoxicam, a commonly used anti-inflammatory agent, associated with carnitines can improve sperm parameters and results are statistically significant. Unfortunately, when discontinuing treatment, parameters come back to baseline [54].

Selenium, required for spermatogenesis and essential for testis development, exerts its antioxidant action through glutathione peroxidase enzymes [58]. Varicocelized rats with altered sperm quality parameters and damage in testicular architecture after administration of sodium selenite significantly improved parameters, and histopathological studies further confirmed the protective effects of sodium selenite [59].

Vitamin E, with a dose-dependent action, is able to scavenge free radicals. In particular, levels of superoxide, H_2O_2, and hydroxyl radicals can be restored by reducing oxidative stress [60]. A dosage of 100 mg, 3 times a day for 6 months, is suggested by Suleiman et al. to decrease lipid peroxidation and to improve pregnancy rate (21% increase in comparison with placebo) [61]. A complex study conducted on varicocelized wistar rats analyzed the effect of vitamin E alone or in association with testosterone on sperm parameters and DNA integrity. Authors reported that the protective effects of

vitamin E and testosterone may be mediated by the enhancement of testicular antioxidant status and upregulation of endocrine activities, which enhanced the Hsp70–2 chaperone expression [62].

Vitamin C, a chain-breaking antioxidant similar to vitamin E, found intracellularly and extracellularly, is essential in keeping the correct balance between oxidants and antioxidants. It neutralizes free radicals, recycles vitamin E, and exerts an action against free radicals damaging DNA [60]. A dosage of 200 mg, daily for 2 months, in association with vitamin E and glutathione has been reported by Kodama et al. to be effective in increasing sperm count and reducing 8-OHdG [63]. Vitamin C effect has been even proved effective in patients with varicocele. Cyrus et al., in a DBPC study, administered 250 mg twice daily to patients after varicocelectomy surgery, demonstrating better sperm parameters in comparison with placebo group [64].

Zinc also has an antioxidant role, particularly when associated with vitamin E. It acts by maintaining a correct ROS regulation and improving sperm concentration, percentage of increasing motility, and consequently pregnancy rate [58]. Association of zinc with *pentoxifylline* and *folic acid* is another possible way to obtain an increase in sperm parameters in patients suffering from varicocele-associated male infertility. In particular, morphology of sperm has increased and results are maintained even 1 month after therapy suspension [65]. Zinc and folic acid have been compared with surgery. Two groups of patients with varicocele have been treated with surgery only or surgery plus supplementation. Combination therapy has 82% of pregnancy rate increase in comparison with 50% increase with surgery only [66].

Vitamins are safe in general but overdosage can be dangerous. Overdose of vitamin C or zinc could cause nausea, diarrhea, and stomach cramps. Overdose of selenium could lead to hair loss, gastrointestinal upset, fatigue, and mild nerve damage [49, 58, 64].

Coenzyme Q10 is a nonenzymatic antioxidant that protects cells against peroxidative damages through low-density lipoproteins [67]; its levels showed a significant correlation with sperm count and motility. Oxidative stress decreases coenzyme Q10 availability in oxidative phosphorylation. Furthermore, coenzyme Q10, present on sperm midpiece, prevents vitamin E prooxidant activity. These data have been reported in a study which has demonstrated the efficacy of coenzyme Q10 in the medical treatment of patients with low-grade varicocele [68]. Another trial reported that the administration of coenzyme Q10 to men with idiopathic asthenozoospermia results in an increase in sperm motility and concentration [69].

GSH and *NAC*, precursor of GSH, have been investigated as possible treatments. Griveau et al. reported that 10 mmol l^{-1} of GSH have an action against DNA damage induced by ROS [70]. Baker et al. observed a protective effect of GSH against sperm impairment and reported increase in sperm motility by activated polymorphonuclear leukocytes [71]. But NAC decreased ROS level and DNA damage and increased sperm motility [72, 73]. Another trial reports that an association of NAC (600 mg daily) with selenium (200 mg daily) is able to increase sperm count, motility, and morphology after 6 months of treatment [74].

Kallikrein exerts a different action, but is capable of improving intratesticular testosterone and increasing sperm maturation. Micic et al. administered 600 units daily for 3 months and reported, an increase in sperm motility (from 24% to 35%) and morphology (from 58% to 71%) in patients with OAT and varicocele [75].

Bioflavonoids, the most important plant pigments for flower coloration, may also act as chemical messengers, physiological regulators, and cell cycle inhibitors. They could improve venous tone in varicocele, increasing mechanical tension, contractile system calcium sensitivity, and peripheral norepinephrine activity. With cyclical therapy of semisynthetic bioflavonoid derivatives, a slower rate of progression from subclinical to palpable varicocele and a higher resolution rate have been reported by Zampieri et al. in adolescent boys. Authors reported that treatment with bioflavonoids is not associated with relevant side effects [76]. Kilic et al. reported that the bioflavonoid micronized purified flavo-

noid fraction (MPFF) is able to relieve varicocele-associated pain and improve all Doppler ultrasound parameters, even if sperm quality did not change [77]. An increase in dosage (1000 mg daily) has been reported by the same authors to be effective even on sperm motility [78]. Tribulus terrestris (TS), another commonly used bioflavonoid, has been extensively studied for varicocle treatment. To date, its role in male infertility is still controversial and needs future double-blind placebo-controlled studies that deploy larger cohorts [79]. Another important trial has been conducted on rats with induced varicocele, and different parameters of oxidative stress have been evaluated (MDA, MMP-2, MMP-9, and TIMP-1). Treatment with (MPFF) has been conducted and results are in favor of treatment [80]. Considering that bioflavonoids are found in certain fruits, vegetables, and other foods like dark chocolate and wine, they are safe and do not have many side effects.

Antioxidants have been mixed in several formulation and results are variable. To date, the perfect formulation has still not been established, and it is difficult to give suggestions.

A complete formulation with L-carnitine; coenzyme Q10; vitamin C, E, B9, B12; zinc; and selenium has been evaluated from a Spanish group to estimate sperm DNA integrity in patients suffering from grade I varicocele. Results are based on semen parameters plus DNA fragmentation: 22.1% reduction in sperm DNA fragmentation and 31.3% fewer highly degraded sperm cells have been reported [81]. Busetto et al. in 2018 in a single center, randomized, double-blind, placebo-controlled trial investigated the effect of 6 months of supplementation with L-carnitine, acetyl-L-carnitine, fructose, citric acid, selenium, coenzyme Q10, vitamin C, zinc, folic acid, and vitamin B12 on sperm quality in 104 subjects with OAT with or without varicocele. Total sperm count increased in supplemented patients, together with a higher progressive and total motility. All of these parameters are, in general, more evident in those suffering from varicocele. Pregnancy rate, has been recorded as a secondary endpoint. Out of 12 pregnancies occurred, 10 were in the supplementation group and only 2 in the placebo group [49].

Lombardo et al. focused almost on all antioxidant agents available on the market [82]. They analyzed every single study per single agent and concluded that, in general, an improvement on the semen was observed with different antioxidants. Those antioxidants that notably increased sperm parameters are vitamin A, vitamin E, and carnitines. Furthermore, in men with low selenium status, the addition of selenium is crucial. They stressed, once again, the importance of a small amount of ROS for capacitation and acrosome reaction while a strong reduction in concentration might have a negative effect. On the other hand, an improvement in semen parameters does not mean an increased fertility. Thus, analyzing all these considerations, it was concluded that the primary outcome of any study should be pregnancy rate. On the other hand, pregnancy rate of an infertile couple may be influenced by multiple confounding factors. Large randomized controlled studies are essential in eliminating potential biases.

Another important limitation that was found in literature was the shortage of double-blind placebo-controlled studies (DBPC) with well-established inclusion/exclusion criteria and with a satisfactory number of patients. Several trials on this topic have been published, with limited quality. Correlation between sperm quality and pregnancy rate was rarely reported, improvement in semen parameters was the surrogate endpoint which was commonly reported.

In conclusion, antioxidants are not only able to prevent reduction in sperm parameters but also able to increase these parameters, particularly improved motility. DNA damage can be treated with antioxidants, and these substances showed decrease in DNA fragmentation induced by oxidative stress.

Considering assisted reproductive technologies (ARTs), cryodamage due to inadequate sperm freezing or improperly conducted thawing procedures can be the cause of sperm damage and can be prevented with supplementation. During ART procedures, OS can be important and several clinical implications are reported. There are evi-

dence that ROS levels are directly connected with ART success [83]. Antioxidants, in particular, when correctly administered, may improve pregnancy rate after these techniques [84].

Hormonal Therapy (Table 15.2)

Hormones, and in particular FSH, are crucial for a correct spermatogenesis. Traditionally, *FSH* is administered for hypogonadotropic hypogonadism but its use has been extended to OAT patients. FSH action on Sertoli cells led to better spermatogenesis with improved sperm-oocyte interaction and may improve pregnancy rate [85]. Unfortunately, results are not conclusive since a number of trials report no benefits with the therapy [86]. In general, it was concluded that FSH may be effective only in patients with OAT and low levels of FSH, but with inhibin B levels in the correct range. Foresta et al. reported that, in those subclasses of patients, the efficacy is confirmed by the statistically significant difference in pregnancy rate between controls and treated patients [87]. Radicioni et al. conducted a trial on patients with confirmed varicocele and FSH was administered for 3 months (75 IU thrice weekly). They reported an increase in semen parameters such as sperm density, total sperm number, forward motility, and atypical forms [88].

Clomiphene and *tamoxifene* have been successfully used to treat male infertility associated with varicocele. In a study, varicocelectomy has been compared to 50 mg daily of clomiphen, and no difference in terms of fertility has been reported between the two options [89]. Tamoxifen in a dosage of 20 mg per day for 6 months registered an increase in sperm concentration in a group of patients with clinical varicocele [90].

The gonadotropin *menotropin (hMG)* has been studied in patients with varicocele, and results have been reported in comparison with surgery or in association with surgery. De Rose et al. conducted a study on 60 patients who were randomized in three groups: patients treated with menotropin, patients treated with menotropin but started the treatment 3 months after surgery, and patients treated only with varicocelectomy. Results reported a significant improvement of sperm parameters in the two groups treated with menotropin with respect to the group treated with only varicocelectomy [91].

Chinese Medicine (Table 15.3)

Chinese medicine is based on several products acting as antioxidants and anti-inflammatory.

Escin is a supplement with anti-inflammatory action that is used in common clinical practice to reduce edema after trauma, inflammation, or after surgical procedures. It has been even studied on infertility. Fang et al. reported that 60 mg of escin every day for 2 months can improve motility and density on patients with varicocele. They concluded that results with escin are inferior to surgery, but significantly superior to controls [92]. Another experience in rats with experimentally induced varicocele reported an increase in sperm counts of the escin-treated groups [93].

The herb *qianjing* should be able to improve sperm quality through increase in epididymal sperm maturation and malondialdehyde effect decrease [94].

Table 15.2 Hormonal therapies

Element	Action	Evidence	References
FSH	Action on Sertoli cells leads to better spermatogenesis.	Not conclusive results. Effective only on patients with OAT and low FSH levels. Other evidence: Improvement in sperm parameters in patients with varicocele.	[85–88]
Clomiphene and tamoxifene	SMER (selective modulator of estrogen receptors).	Improve sperm parameters in patients with varicocele.	[89, 90]
Menotropin (hMG)	Hormonally active agent, usually a mixture of gonadotropins.	Improves sperm parameters in patients with varicocele.	[91]

Table 15.3 Chinese medicine

Element	Action	Evidence	References
Escin	Anti-inflammatory and anti-edema action.	Effective in patients with OAT with or without varicocele. Improve sperm parameters and pregnancy rate.	[92, 93]
Qianjing	Antioxidant effect on hormone modulation.	Increase in glutathione peroxidase and decrease of malondialdehyde.	[94]
Guizhi Fuling Wan	Activator of blood circulation and reduces stasis.	Improves sperm parameters in patients with varicocele.	[95]
Green tea	Antioxidant effect through polyphenols action.	Decrease of apoptosis of spermatogenic cells.	[96]
Other Chinese substances (Wu-Zi-Yan-Zong-Wan, Fu pen Zi, Ba Ji Tian, Tu Si Zi, etc.)	Action on kidneys, cause of blood stasis.	Still not possible to recommend; still not enough evidence.	[97]

Guizhi Fuling Wan is able, as well, to increase sperm parameters on patients with male infertility secondary to varicocele [95].

Tea, and in particular *green tea*, is rich in polyphenols: epigallocatechin gallate (EGCG), epigallocatechin, epicatechin gallate, epicatechin, kaempferol, quercetin, and myricitin. A Chinese group conducted a study on rats with varicocele and found that tea phenols are able to reduce the apoptosis of spermatogenic cells in a dose-dependent manner [96].

Several other *Chinese herbal substances* (Wu-Zi-Yan-Zong-Wan, Fu Pen Zi, Ba Ji Tian, Tu Si Zi, etc.) have been proposed as possible treatments for infertility following varicocele. For traditional Chinese medicine, kidney is the essential organ that stores and plays a crucial role in reproduction. In particular, they think that kidney emptying and blood stasis are basic causes of varicocele infertility and therapy should be based on supplementing this organ and promoting blood circulation. An interesting meta-analysis on Chinese herbal medication reports that on the basis of actual evidence, it is not possible to recommend such therapies because the current evidence is of insufficient quality [97].

Recently, there has been an important growth in knowledge on male infertility and sperm activity. Though diagnostic tools have been improved, therapies didn't develop enough. To date, there are still unclear targets, varying drug combinations, inadequate outcome measures, and poor evidence coming from small studies not well designated [98]. In accordance with guidelines of different urological/andrological societies, varicocelectomy is the treatment of choice for OAT secondary to varicocele. Drugs alone cannot be suggested for varicocele based on current evidence. The role of medical therapy to hasten restoration of seminal parameters after surgery, or in those who has no improvement in semen parameters after surgery should be explored.

Future Perspectives

In a near future, interest could be focused on molecular and genetic factors to better understand all the mechanisms related to varicocele damages and effects. Furthermore, this could represent a good chance to identify new targets for different medical therapies. It is possible that preexisting genetic lesions or defects in molecular pathways can play a role in testicular injury due to varicocele. For example, heat-shock proteins (HSP), released during heat stress, have a protective effect on cells. Studies reported that HSPA2 is downregulated in sperm of patients with varicocele [99] and that gene expression of HSFY is higher [100]. Another possible target that is directly connected with varicocele is the oxidative stress, but before understanding its role it is important to find correct strategies to reach a correct measure and understand balance between oxidants and antioxidants

[101]. Romeo et al. evaluated nitrotyrosine concentration as a marker of nitric oxidative damage and reported a statistically significant difference between adolescent with and without varicocele [102]. Cervellione et al. studied basal thiobarbituric acid reactive substances (TBARS) to measure lipid peroxidation and found a direct correlation with varicocele [103].

The role of microRNAs (mi-RNA) in spermatogenesis has been studied in accordance with their function on cell proliferation, differentiation, and apoptosis. An important association between miR-15a and its target HSPA1B with varicocele has been found. HASPA1B mRNA showed a significantly increase in patients whereas miR-15a revealed a significant decrease. This mechanism plays a role in promoting cell survival while decreasing cellular stresses and sperm can be protected from hyperthermia and oxidative stress [104]. In conclusion, mi-RNAs, but even any other molecular pathway, could be a novel biomarker and a potential therapeutic target in future to handle male infertility related to varicocele.

In a modern view, sperm provide a specific epigenetically marked DNA, a complex population of proteins, and RNAs required for embryogenesis. For these reasons, −omic technologies (proteomics, epigenomics, etc.) should develop and start considering male semen much more than a simple medium to carry the spermatozoa through the female reproductive tract [105].

Conclusions

Several medical approaches have been studied for varicocele treatment. To date, much evidence is still not available to develop an effective treatment. More DBPC studies evaluating the best regimen of medical treatment and considering the effect of standardized doses in large trials are required. Due to this reason, guidelines are still lacking conclusive suggestions for varicocele treatment. Conducing big trials is the only way to obtain a better knowledge on medical therapy and to establish the real efficacy of these treatments.

Review Criteria

An extensive search of studies examining the relationship between varicocele and medical therapy was performed using search engines such as ScienceDirect, Google Scholar, PubMed, and MEDLINE. The start and end dates for these searches were April 2018 and October 2018, respectively. The overall strategy for study identification and data extraction was based on the following key words: “varicocele”, “varicocele medical treatment”, “antioxidants”, “hormonal agents varicocele”, “Chinese medicine”, “oxidative stress”, “reactive oxygen species”, “infertile men”, “varicocelectomy”, “infertility”, “semen parameters”, and “pregnancy rate” as well as the names of specific medical agents. Data that were solely published in conference or meeting proceedings, websites, or books were not included. Websites and book chapter citations provide conceptual content only.

Multiple Choice Questions and Answers

1. Which of the following is NOT a medical treatment for varicocele?
   (a) Chinese medicine.
   (b) Hormonal agents.
   (c) Antioxidants.
   (d) **Chemotherapy**.
2. Regarding reactive oxygen species (ROS):
   (a) **The correct balance should be maintained between production and clearance**.
   (b) Should be as less as possible.
   (c) It is not an important parameter to take into consideration.
   (d) Should be as much as possible.
3. Which of the following is NOT commonly used for male infertility treatment?
   (a) Carnitines.
   (b) Coenzyme Q10.
   (c) **Cranberry**.
   (d) Selenium.

4. Medical therapy for varicocele:
   (a) Is more effective than varicocelectomy.
   (b) **Is still under debate and international guidelines are still inconclusive**.
   (c) Is an alternative to varicocelectomy with same outcomes.
   (d) It is required only before varicocelectomy.
5. Which is/are the current focus on medical therapy development for male infertility?
   (a) Heat-shock proteins (HSP).
   (b) MicroRNAs (mi-RNA).
   (c) Genomics and proteomics.
   (d) **All the above**.

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Macroscopic Surgical Techniques for Varicocele Repair

16

Sarah C. Krzastek, Connor Rotterman, Ryan P. Smith, and Jason R. Kovac

Abbreviations

ART	Assisted reproductive techniques
AUA	American Urological Association
US	Ultrasound

Key Points

- Microsurgical varicocele repair is of historical importance.
- Microsurgical varicocele repair is the current gold standard.
- Microsurgical repair can be performed via an inguinal or a subinguinal approach.
- Laparoscopic varicocele repair is associated with increased complications compared to microsurgical repair.
- Varicocele repair can be conducted concurrently with hernia repair and vasectomy.

Introduction

A varicocele is defined as an abnormal dilation and tortuosity of the testicular pampiniform complex of veins. Varicoceles may be present in 15% of the general population and have been associated with male-factor infertility, failure of testicular growth and development, testicular atrophy, and chronic scrotal pain. Repair of the varicocele has been shown to improve these parameters [1–4]. Historically, varicocele repair was performed to treat scrotal pain. The conventional surgical management of varicoceles was developed in the early twentieth century by Ivanissevich [5]. His study of cadavers led to the theory that varicoceles develop as a result of incompetent venous valves and venous reflux, and he proposed the technique of identifying the spermatic cord through an inguinal incision, with ligation of the varicose veins at this area [5]. As further studies identified the link between varicoceles and male infertility, the indication for varicocele repair shifted to include treatment of infertility. This reason for repair was first illustrated by Tulloch in the 1950s [5], and since that time, varicocele repair has become the most commonly performed surgery for the treatment of male infertility [6].

Physiologically, varicoceles are thought to impair spermatogenesis and testosterone synthesis via associated heat stress, excess reactive oxygen species, and increased apoptosis of cells within the testis [7]. Varicocele repair has been

S. C. Krzastek · R. P. Smith
Department of Urology, University of Virginia, Charlottesville, VA, USA

C. Rotterman · J. R. Kovac (✉)
Men's Health Center, Indianapolis, IN, USA
e-mail: jkovac@urologyin.com

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shown to improve both semen parameters and testosterone production in hypogonadal males with subfertility [8, 9]. Varicocele repair in azoospermic and oligozoospermic patients improves pregnancy rates and live birth rates following the use of assisted reproductive techniques (ART), even if azoospermia or oligozoospermia persists [10]. Additionally, varicocele repair has the potential to eliminate the need for ART altogether [11].

Dubin and Amelar developed a grading system in the 1970s for varicoceles, ranging from subclinical to Grade III, which is still used today [12]. A subclinical varicocele is noted incidentally on scrotal ultrasound (US) and is not palpable or visible on clinical exam. Grade I varicoceles are not visible on exam and are only palpable with Valsalva. Grade II varicoceles are not visible, but are easily palpable on exam without Valsalva pressure, and Grade III varicoceles are visible without Valsalva. According to the American Urological Association (AUA) Male Infertility Best Practice Policy Committee, indications for varicocele repair include couples attempting conception with documented infertility in which the male partner has one or more abnormal semen parameters and a palpable varicocele. Varicocele repair is not currently indicated for patients with normal semen parameters or a subclinical varicocele [13].

Multiple approaches have been taken for the repair of the varicocele over the years and range widely from antegrade/retrograde embolization to surgical ligation. Figure 16.1 illustrates the incisions made for the various approaches to varicocele repair. Microsurgical varicocele repair is

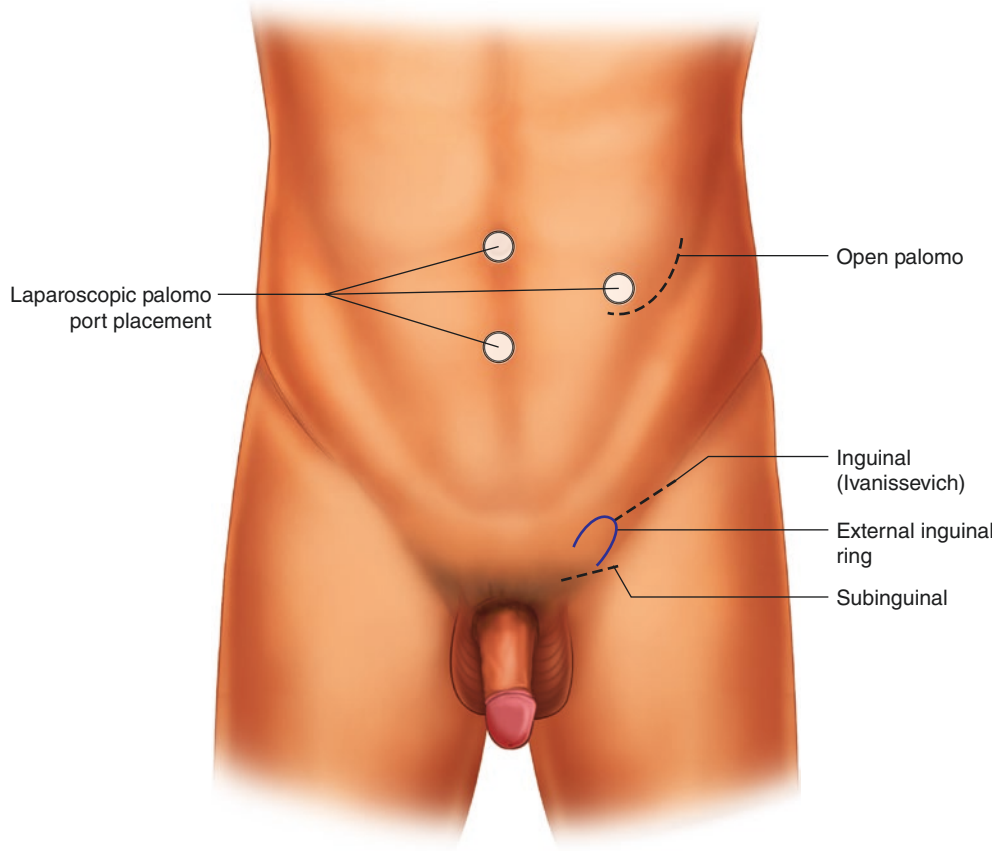

Fig. 16.1 Incision locations for varicocele repair, via the subinguinal approach, open Ivanissevich inguinal approach, and Palomo technique (laparoscopic and open)

currently the gold standard of treatment. However, surgical repair of the varicocele may also be performed macroscopically, either laparoscopically via a transabdominal approach, or open via a retroperitoneal (Palomo), inguinal (Ivanissevich), or subinguinal approach. The aim of this chapter is to summarize macrosurgical varicocele repair in general, while focusing specifically on the open inguinal and subinguinal approaches. Although these repairs are not currently classified as the gold standard, an understanding of these historical approaches to varicocele repair is beneficial to guide current management.

Open Varicocele Repair (Inguinal and Subinguinal Approaches)

The open macroscopic varicocele repair remained the standard of care until the advent of modern microsurgery in the 1970s. In the early 1980s, several groups reported on the rate of arterial injury during varicocelectomy and suggested the implementation of optical magnification to minimize arterial and lymphatic complications, using either loupes or the operating microscope [5, 14, 15]. While the use of the operating microscope maximizes the visualization of arterial and lymphatic structures, loupes allow for adequate visualization without the learning curve required for the use of the microscope and microsurgical instruments.

The traditional inguinal open varicocele repair (Ivanissevich technique) is started by making an incision in the inguinal region above and lateral to the ipsilateral pubic tubercle and extending laterally along the skin lines of the inferior abdominal wall. While techniques and approaches can differ, one approach is detailed here. To start, the aponeurosis of the external oblique muscle is sharply incised along the length of the fibers to open the inguinal ring and expose the spermatic cord. Once the spermatic cord is identified, the cord is grasped with a Babcock clamp and isolated from surrounding tissues with the assistance of a kittner sponge. A tongue depressor or metal ruler covered with a penrose drain is placed under the isolated cord to act as a backdrop for the operation. The external spermatic fascia is then incised to access the vascular structures within. The vas deferens and vasal artery should be identified and preserved. Venous structures, including the internal spermatic vein, cremasteric veins, external spermatic veins, gubernacular veins, and periarterial veins, have all been described to be part of the body of varicoceles and should be identified and dissected, or ligated [16]. Any arteries and lymphatic vessels should be clearly identified and preserved to avoid complications.

The approach during a subinguinal varicocelectomy is similar, except that the initial skin incision is made at the level of the external inguinal ring to allow delivery of the spermatic cord without dividing the muscles or fascia of the abdominal wall. This approach is less painful and has less morbidity compared to other open varicocelectomy techniques. However, the more distal the approach, the more the vessels have branched, resulting in smaller vessel diameters that require ligation. Therefore, the use of optical magnification with loupes or the operating microscope is required when performing this technique to allow for adequate identification of venous structures and avoidance of arterial and lymphatic vessels [16]. Studies have also shown that the use of the microscope increases the number of correctly identified venous, arterial, and lymphatic branches during an inguinal approach [17].

Though the pampiniform plexus of veins may be accessed through a scrotal incision, this approach should be avoided. At the scrotal level, the veins are highly branched and closely associated with the testicular artery, which significantly increases the risk of damage to the arterial supply to the testis during vein ligation and may result in further testicular atrophy and fertility impairment [6].

Traditional non-magnified inguinal varicocelectomy has a lower varicocele recurrence rate than a retroperitoneal approach (described below). However, the rates of testicular artery injury and hydrocele formation remain high (compared to the microsurgical approach) with hydrocele rates typically ranging from 3% to15% [6, 18]. A 2009 meta-analysis of 36 studies

assessed the rate of spontaneous natural pregnancy, varicocele recurrence, and hydrocele formation among varicocele repair performed via the open retroperitoneal Palomo technique, laparoscopic technique, radiologic embolization, and macro- or microscopic repair [19]. Of the various approaches, the macroscopic and microscopic inguinal and subinguinal repairs had the highest natural pregnancy rates and the lowest recurrence rates [19]. The rates of natural pregnancy were 42% following microscopic repair as compared to 36% following macroscopic repair. Recurrence rates were 1.1% versus 2.6%, respectively. The rate of hydrocele formation following macroscopic repair approached that of the Palomo technique (7.3% versus 8.24%, respectively), which was much higher than following the microscopic repair (0.4%) [19]. The use of loupe magnification had a lower rate of hydrocele formation and recurrence compared to non-magnified repair (2.9% v 5.9% hydrocele rate, 2.9% v. 8.8% recurrence rate, respectively). These data suggested that the loupe-magnified macroscopic repair has similar outcomes to the microscopic repair, though with a higher rate of hydrocele formation postoperatively [19]. Table 16.1 summarizes the data synthesized by Cayan et al.

It has been shown that identification of key anatomic structures is improved with the use of the microscope, as compared to the use of loupes [20]. However, loupe magnification is superior to standard open subinguinal varicocelectomy without magnification [21, 22] and may be an appropriate alternative in facilities without an operating microscope, or for the urologist without significant experience using the operating microscope.

High Retroperitoneal and Laparoscopic Varicocele Repair

Varicocelectomy may also be performed using the Palomo technique, either through an open or laparoscopic approach. These techniques are more commonly employed in the pediatric population. The traditional open retroperitoneal varicocele repair, or open Palomo technique, was first described by Palomo in 1949 [23]. During Palomo's study of the vascular anatomy, it was concluded that if one of the three arterial supplies to the testis was spared during surgery, the testis would remain viable. While it has been shown that inadvertent ligation of the testicular artery during surgery may not have a negative impact on semen parameters [24, 25], other studies have suggested that ligation of the testicular artery results in damage to the seminiferous tubules [26]. It is the authors' opinion that maximal preservation of arterial flow to the testicle be attempted during any approach to the varicocelcectomy.

In Palomo's initial series, a 4-cm incision was made 3-cm above the internal inguinal ring (Fig. 16.1). At this location, the large spermatic veins and testicular artery were ligated together,

Table 16.1 Comparison of outcomes and common complications associated with various surgical approaches to varicocelectomy

Surgical approach	Rate of hydrocele formation	Rate of varicocele recurrence	Rate of spontaneous pregnancy
Inguinal			
Macroscopic	7.3%	2.6% (0–37%)	36% (34–39)
Microscopic	0.4% (0–0.7%)	1% (0.6–4%)	42% (37–56%)
Subinguinal			
Macroscopic (Ivanissevich)	7.3%	2.6% (0–37%)	
Microscopic	0.4% (0–1.6%)	1% (0–3%)	42% (33–51%)
Retroperitoneal (open Palomo)	8% (6–10%)	15% (7–35%)	38% (25–55%)
Laparoscopic (lap Palomo)	2.8% (0–9.4)	4.3% (2.2–7.1%)	30% (16–40%)
Radiologic embolization	NA	12.7% (2–24%)	33% (21–40%)

Based on data from Ref. [19]

with care taken to spare the cremasteric and deferential arteries. Currently, the open Palomo technique ligates the internal spermatic vein between the anterior superior iliac spine and the renal vein, usually through a Gibson incision. This approach is technically more challenging than the open inguinal or subinguinal approaches, as the structures are deeper and more difficult to visualize, which increases the rate of hydrocele formation [18]. In a meta-analysis by Cayan et al., the open Palomo technique was associated with a hydrocele rate of 8.24%, with a reported 15% recurrence rate [19]. Recurrence and failure rates were unfortunately high if the testicular artery was preserved since this artery was associated with a periarterial venous plexus, which may dilate following ligation of collateral veins [27].

The Palomo technique may also be performed laparoscopically. This is the preferred technique for varicocele repair in the adolescent population, as the open approach has largely fallen out of favor given the benefits of minimally invasive surgery. Varicocele repair should be considered in the adolescent population in the setting of decreased ipsilateral testicular volume [28]. Laparoscopic varicocele repair typically uses three transperitoneal ports. The abdomen is insufflated using a Veress needle or Hassan technique, and a 5 mm port is placed at the umbilicus, with an additional 5 mm port at the midline between the umbilicus and pubic symphysis, and a third 5 mm port lateral to the ipsilateral inferior epigastric vessels. Single site laparoscopic varicocelectomy has been performed in some centers and has been shown to decrease postoperative pain and shorten recovery time, with no adverse impact on semen parameters [29]. The peritoneum is opened 3 cm proximal to the internal inguinal ring. The spermatic vessels are dissected from surrounding tissues with the assistance of the Doppler. The veins are then ligated with clips and divided [30]. The testicular artery may or may not be spared. Similarly to the open Palomo technique, sparing the testicular artery has been associated with higher recurrence rates [31], though ligating the artery has been associated with slower catch-up testicular growth rates when performed in adolescents [32]. Other studies comparing the open Palomo technique to laparoscopic varicocelectomy have shown similar results. One recent retrospective review showed similar recurrence rates and rates of hydrocele formation, with slightly higher recurrence rates with the artery-sparing open approach and slightly higher hydrocele rates with the laparoscopic approach, which is consistent with prior studies [33]. The majority of studies suggest a recurrence rate of 3–6% and hydrocele rate of 7–50% based on the technique used [30, 34].

Concurrent Surgical Procedures

Vasectomy

A varicocele may be incidentally identified in men presenting for vasectomy. Interestingly, some studies suggest that early identification of varicoceles in men presenting for vasectomy may aid in early identification of patients at risk of hypogonadism as varicoceles have been associated with hypogonadism. This would facilitate early treatment of hypogonadism in at-risk men [35]. If a varicocelectomy is being considered in patients undergoing vasectomy, special attention must be paid to preservation of the deferential vessels, as the testicle may become reliant on these vessels for blood supply following varicocele repair. These vessels are easily injured during a traditional non-microscopic vasectomy. However, concurrent vasectomy and varicocele repair can be performed safely in patients with clinically significant varicoceles and testicular pain or asymmetry, with the use of the operating microscope and Goldstein's technique described in 2007 [36]. During this procedure, a microscopic subinguinal varicocelectomy is performed by ligating the internal spermatic and cremasteric veins. The deferential vessels are carefully dissected off the vas deferens with subsequent completion of the vasectomy. In their initial series of 18 patients who underwent concurrent vasectomy and varicocelectomy, there were no complications or recurrent varicoceles [36].

Hernia Repair

Given the frequency of both hernias and varicoceles, concurrent pathology is possible, and it is conceivable that these may be repaired during the same operation. Additionally, the urologist's intimate knowledge and understanding of the inguinal anatomy make him uniquely suited for hernia repairs. Alternatively, general surgery can be consulted to perform the hernia repair. Concurrent laparoscopic varicocelectomy and hernia repair have been described in the pediatric literature [37]. Additionally, an open approach to the combined inguinal hernia repair and varicocelectomy has also been described [38, 39]. Schulster et al. described a technique in which the skin is incised in standard fashion for an inguinal hernia repair and the spermatic cord is identified at the external ring along with the genital branch of the genitofemoral nerve and ilioinguinal nerve. The aponeurosis of the external oblique is opened, the hernia sac is identified and dissected off the spermatic cord, opened, contents reduced, and sac excised. The spermatic fascia is then opened and the varicocelectomy is performed as previously described. The spermatic fascia is closed, and the hernia defect is repaired with mesh. This surgery is performed with the use of the operating microscope, which allows for clear identification of vascular and nervous structures and prevents nerve entrapment [38]. In their series of 291 microscopic inguinal hernia repairs, concurrent varicocelectomy was performed in 56%. The addition of varicocele repair added approximately 60 minutes to the operating time. With a median follow-up of 8.6 months, none of their patients developed postoperative pain or sensory loss, there were no hernia recurrences, and there was only one varicocele recurrence [38] This suggests that not only can the rate of complications following inguinal hernia repair be significantly reduced with the use of the operating microscope, additional scrotal surgeries can be completed in a safe and effective fashion within the same operation.

Controversies

Use of the operating microscope may increase the number of correctly identified vascular and lymphatic structures and reduce postoperative complications including hydrocele formation and varicocele recurrence. With the expansion of microsurgical expertise, some would argue there is no longer any indication for a macroscopic varicocele repair in the setting of fertility preservation. While the microscopic varicocelectomy is currently the gold standard for management of the disease, the macroscopic repair remains a way to manage the clinically significant varicocele. However, given the current literature and understanding, referral of patients with varicoceles should be made to an andrologist with sufficient microsurgical skills to perform the varicocele repair microscopically.

Conclusions

The macroscopic varicocele repair remains a highly effective and viable approach to the management of the clinically significant varicocele, with the best outcomes seen with an inguinal or subinguinal approach. The macroscopic approach has the added benefit of avoiding the learning curve associated with the operating microscope and microsurgical instruments. The use of the operating microscope is not required allowing the procedure to be conducted in remote centers. However, a microsurgical approach is preferred since it increases the number of correctly identified vascular and lymphatic structures, while reducing postoperative complications and improving outcomes.

Review Criteria

An extensive search of manuscripts and resources was conducted using a multitude of approaches. The main source of studies and data obtained was using search engines

such as Google Scholar, PubMed, and MEDLINE. The primary strategy was to identify initial studies and review papers using the key words "varicocele", "varicocelectomy", "repair", "surgery", and "macroscopic". From there, specific resources were identified in the same search engines using the following key words: "open", "inguinal", "subinguinal", "Ivanissevich", "pampiniform", "retroperitoneal", "laparoscopic", "Palomo", "concurrent", "vasectomy", and "hernia". High-impact and relevant critical review papers were obtained and reviewed individually. Their observations provided conceptual content only. The review papers were analyzed and their cited references were examined. In select cases, these references were obtained and reviewed directly to ensure proper results were included in the current report. Articles published in languages other than English were not considered. Data published solely in conference proceedings and random websites were not included.

Multiple Choice Questions and Answers

1. Varicoceles are associated with
   (a) Male-factor infertility.
   (b) Failure of testicular growth/development.
   (c) Chronic scrotal pain.
   (d) **All of the above**.
2. Physiologically, varicoceles impair spermatogenesis and testosterone synthesis via
   (a) Heat stress.
   (b) Excess reactive oxygen species.
   (c) Increased testicular apoptosis.
   (d) **All of the above**.
3. A Grade 2 varicocele is
   (a) Subclinical and noted incidentally on scrotal ultrasound.
   (b) Not visible on exam, and only palpable with Valsalva.
   (c) **Not visible, but are easily palpable on exam without Valsalva pressure**.
   (d) Easily visible without Valsalva.
4. The pampiniform plexus of veins may be accessed through a scrotal incision; however, ligation should be avoided because
   (a) **At the scrotal level, the veins are highly branched and closely associated with the testicular artery, which significantly increases the risk of damage to the arterial supply to the testis during vein ligation**.
   (b) It may interfere with the lymphatic drainage.
   (c) It is too difficult.
   (d) It takes too much time.
5. The current gold standard for surgical repair of grade 3 varicoceles is
   (a) Laparoscopic.
   (b) Open subinguinal with loops.
   (c) Radiographic embolization.
   (d) **Open inguinal/subinguinal approach with microsurgical repair using an operative microscope**.

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Microscopic Surgical Techniques for Varicocele Repair

17

Russell P. Hayden and Marc Goldstein

Key Points

- Appropriate patient selection is critical to the success of varicocelectomy.
- Choice of varicocelectomy technique will depend upon surgeon experience, available resources, and patient characteristics.
- Subinguinal microsurgical varicocelectomy carries the lowest documented failure and complication rates.
- The majority of varicocele recurrences following repair are due to technical error.
- Results rely upon formal microsurgical training, which should not be bypassed.

Introduction

The varicocele represents an aberrant dilation of the pampiniform plexus within the spermatic cord. The incidence of varicocele is commonly quoted to be ~15%, with the majority of affected men demonstrating normal fertility [1, 2]. Most varices develop concurrently with puberty, and thereafter, the observed incidence progressively increases with age [3, 4]. Though most varicoceles remain clinically silent, a correlation with male infertility has long been recognized in a subpopulation of afflicted men, an association which serves as the principle rationale for varicocele repair to improve reproductive potential [5]. The practice remains controversial, however, since most of the older literature has not addressed live birth outcomes [6, 7].

Though birth rates remain the ideal endpoint for any fertility intervention, this measure is inherently problematic due to the introduction of female factor, as well as coital frequency. The effect of varicocelectomy requires large sample sizes and non-operated controls to adequately assess the intervention [8]. As a result, much of the evidence supporting varicocele repair has relied upon improvements in semen parameters, an intuitive but flawed surrogate for male fertility [9]. Nonetheless, the link between improved semen parameters with a varicocele intervention has been consistently demonstrated [10, 11]. These data support the clinical observations

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R. P. Hayden · M. Goldstein (✉)
Department of Urology, Weill Cornell Medicine, New York, NY, USA
e-mail: mgoldst@med.cornell.edu

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commonly quoted to justify varicocelectomy, which include: a higher perceived incidence among individuals with primary and secondary infertility, return of sperm to the ejaculate in men previously azoospermic, and a dosage relationship relating varicocele grade with poorer semen quality [2, 12–16].

Until recently, the reproductive urologist relied upon studies that were predominately based upon observational data to support the use of varicocelectomy. By 2012, however, enough randomized control trials had accumulated to perform adequate meta-analyses to address the endpoint of birth outcomes [17]. Kroese et al. aggregated 10 studies accounting for 894 men, resulting in a statistically significant improvement in birth rates (odds ratio 2.39, CI 1.56–3.66) when restricting their data to subjects with palpable varicoceles and impaired preoperative semen parameters [18]. This expansion of the evidence was supported by other contemporary studies, with the estimated number needed to treat ranging from 5.2 to 17 [11, 18]. Although these data are far from perfect and allows for continued debate, the argument for varicocele repair in the properly selected patient has never been stronger.

Preoperative Evaluation

Proper patient selection is paramount to success when considering varicocele repair. The indications for varicocelectomy per the major urologic societies are generally in agreement, which includes the American Urological Association (AUA), the American Society for Reproductive Medicine (ASRM), and the European Association of Urology (EAU) [19–21]. Varicocele repair should be considered in men with a palpable varix on physical exam, abnormal semen parameters, and in whom fertility is desired assuming the female partner is fertile or has a treatable infertility diagnosis. Varicocele-related pain is a relative indication, although care must be taken to accurately ascribe the discomfort to the presence of the varicocele [22]. In adolescents, it is agreed that testicular hypotrophy and/or pain is an indication for repair, although the degree of hypotrophy and the timing for intervention are debated. These guidelines provide a reliable means to counsel the infertile man with concomitant varicocele. Of note, isolated teratozoospermia is no longer considered an indication per the most recent rendition of the AUA-ASRM statement [20].

Another relative indication for varicocele repair is androgen deficiency. It is established that varicoceles cause a pan-testicular insult, with impaired Leydig cell function in addition to the dysfunction of Sertoli and germ cell lines [23–25]. Large studies have confirmed the link between varicocele and low serum testosterone, with varicocele repair often improving the testosterone levels in men with worse preexisting testosterone deficiency [2, 26–28]. A subsequent meta-analysis reviewed nine studies totalling 814 subjects [29]. They demonstrated a mean increase of total serum testosterone following varicocelectomy of 97.4 ng/dL (CI 43.7–151.2). These compelling data provide a reasonable indication for varicocele repair in men with coexisting androgen deficiency, a situation that may clinch the decision to treat in otherwise borderline cases. Both the AUA-ASRM joint statement and the EAU have now included language to address the expanding role of varicocele repair in men with low androgen levels [20, 30].

Options for Varicocele Repair

Multiple treatment modalities have been employed for repair of varicoceles. Table 17.1 presents techniques of varicocele repair with success and complication rates. Surgical complications include postoperative hydrocele (secondary to excessive ligation of lymphatics), hematoma, and varicocele persistence and recurrence.

In a large randomized study, Al-Kandari et al. compared traditional open techniques (without microscope assistance) against laparoscopic and subinguinal microsurgical varicocelectomy [31]. A hydrocele developed in 13%, 20%, and 0% of cases in the open, laparoscopic, and microsurgical groups, respectively. The number of recurrences

Table 17.1 Modalities of varicocele repair: classically reported success rates and complications

Treatment	Advantages	Disadvantages	Recurrence rate	Complication rate	Source
Embolization	Minimal pain	Difficult canalization of the right spermatic vein	3.2–19.3%	Extravasation NR Thrombophlebitis NR	Cayan [32] Cassidy [33]
Laparoscopic	Simplified vascular anatomy, short operating time, can address bilateral varices simultaneously	Difficulty visualizing lymphatics	4.3%	Hydrocele 2.8–20%	Al-Kandari [31] Cayan [32]
Microscopic subinguinal	Adequate anatomic visualization, possibly less pain	Complex vascular anatomy	1.05%	Hydrocele 0–0.4%	Al-Kandari [31] Cayan [32]
Microscopic inguinal	Simplified vascular anatomy, adequate visualization	Possibly more incisional pain	2.1%	Hydrocele 0.7%	Cayan [32]
Loupe-assisted high ligation	Simplified vascular anatomy	Incisional pain, poor visualization of structures	14.9%	Hydrocele 8.2–13%	Al-Kandari [31] Cayan [32]

was 7, 9, and 1 in the open, laparoscopic, and microsurgical arms, respectively. Improvements in semen parameters and birth rates were similar across all techniques. In a follow-up meta-analysis encompassing 36 studies, Cayan et al. documented comparable outcomes (see Table 17.1) with subinguinal microsurgical varicocelectomy remaining the best performer [32]. Cayan and colleagues also reviewed the published literature for varicocele embolization, reporting a recurrence rate of 12.7%. A subsequent large series by Cassidy and colleagues provides additional details regarding the success of embolization [33]. When including right-sided attempts in the final analysis, which is significantly more difficult to cannulate for the interventional radiologist, failure rates were comparable to that of Cayan et al. at 19.3% for bilateral varices. However, when narrowing to only left varicoceles, the failure rate drops to 3.2%.

For the patient seeking the least likelihood of recurrence, inguinal or subinguinal microsurgery outperforms image-guided techniques. Given the high initial cost and maintenance of an operating microscope, along with the investment of microsurgical training, multiple groups have studied whether traditional loupe magnification is sufficient [34]. In an early study by Goldstein et al., 2.5× loupes were compared against the operating microscope [35]. Their retrospective review demonstrated a recurrence and hydrocele rate of ~9% with loupe assistance, whereas the microscopic technique was characterized by a 0.6% recurrence rate and an absence of postoperative hydroceles over a course of 640 varicocelectomies. A follow-up study by Cayan and colleagues compared a macro-inguinal approach against the micro-subinguinal repair [36]. Similar to the earlier account, the operating microscope imparted a recurrence rate of 2.1% as opposed to 15.5% in the more traditional technique. Finally, in a unique study by Liu et al., an independent surgeon aided by the operating microscope graded intraoperatively the dissection of a colleague who marked arteries, veins, and lymphatics without magnification [37]. Concerningly, an average of 0.74 arteries were marked for ligation and an average of 2.14 veins would have been missed. These data provide clear evidence that the operating microscope is necessary to perform a high-quality varicocelectomy.

From the above data, it is apparent that the microsurgical varicocelectomy provides the most reliable success rates combined with the lowest reported complications. Additionally, the subinguinal approach appears to limit postoperative pain since the external oblique aponeurosis is

never violated, although there have been conflicting reports [38, 39]. To this end, we consider the subinguinal microsurgical varicocelectomy the current gold standard for repair.

Focused Anatomic Review

The vascular pedicle of the testicle is classically described as one artery and one vein that originate asymmetrically. Both the left and right testicular arteries originate from the aorta; while the left internal spermatic vein drains into the left renal vein, the right inserts directly into the vena cava at a sharp angle. This classical anatomy is only present in 80% of men, with atypical origins and collateralization above the iliac canals being common [40]. Upon entering the inguinal canals, these "solitary" vessels begin to branch, with the internal spermatic vein forming the pampiniform plexus (Fig. 17.1). The vessels feeding the external spermatic fascia typically arise from the infe-

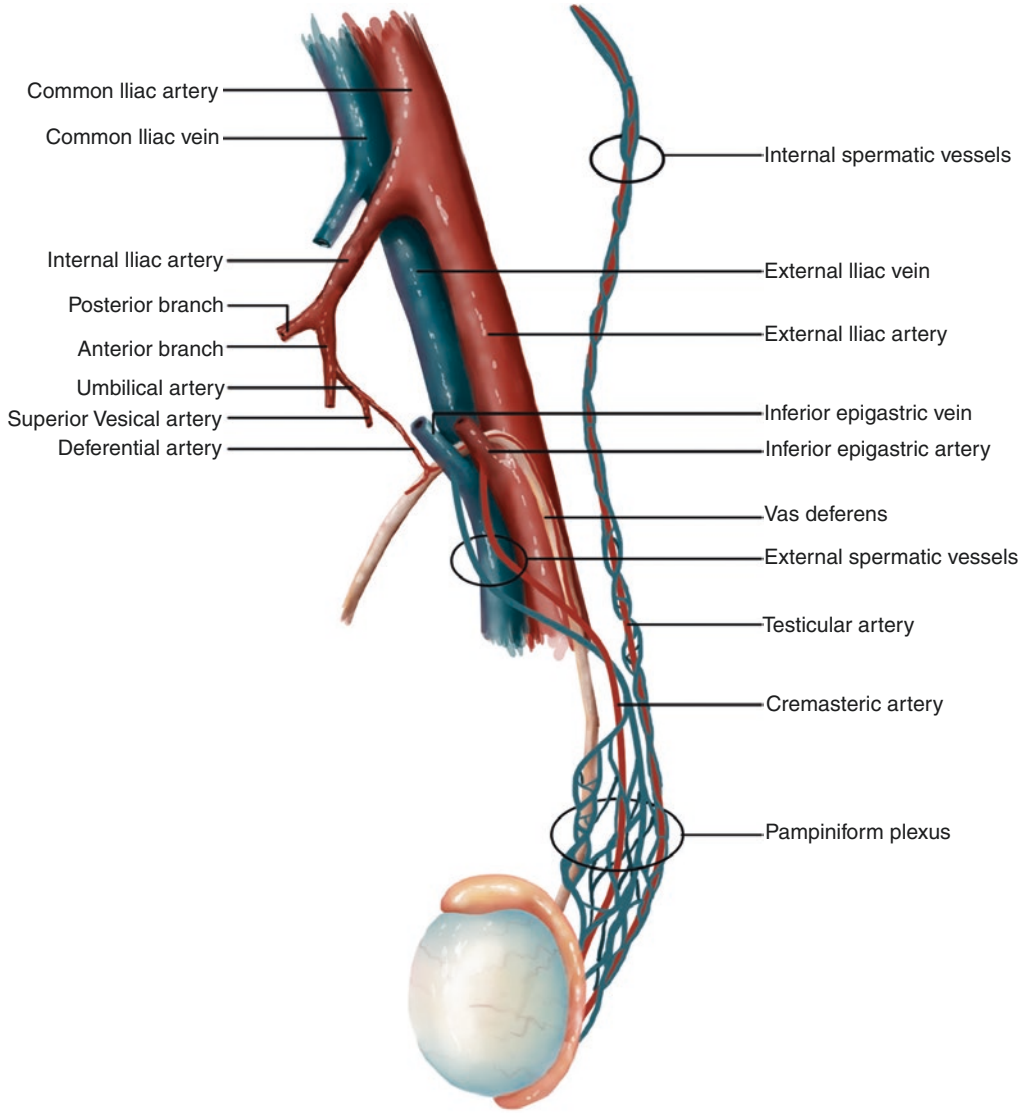

Fig. 17.1 An overview of vascular anatomy of the testis

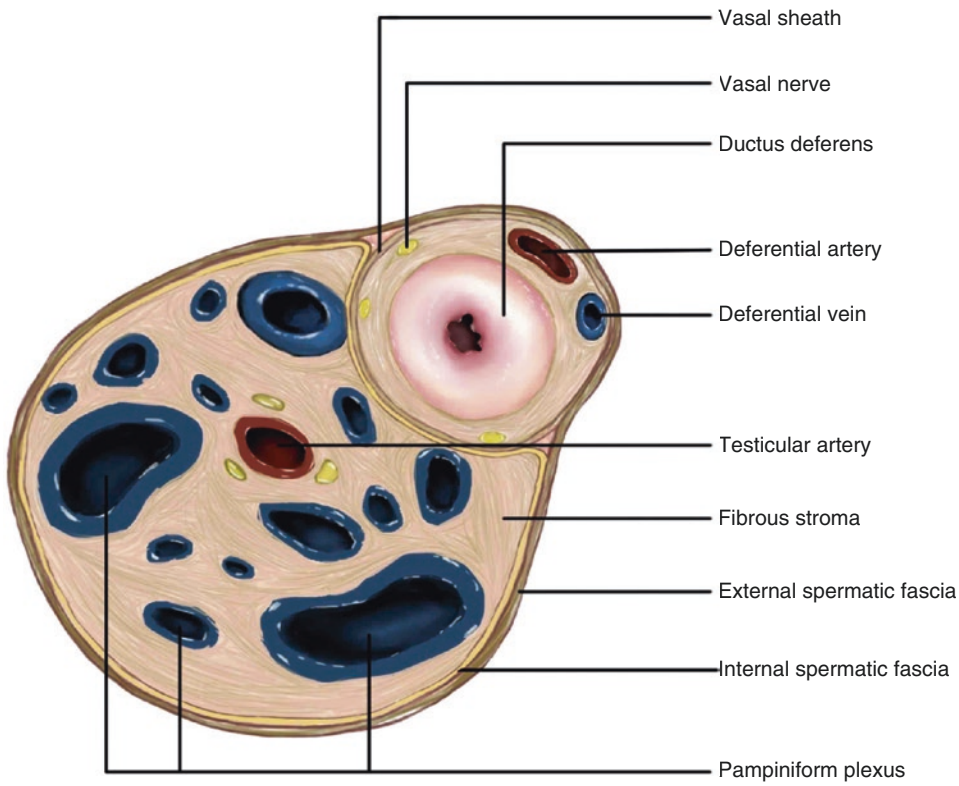

Fig. 17.2 Cross-sectional anatomy of the spermatic cord, demonstrating the location of the vasal sheath between the internal and external spermatic fascia

rior epigastrics, which may have distal collateralization into the internal spermatics [41]. Finally, an alternative venous outlet of the testis lies in the gubernacular veins, linking the testis circulation with the general scrotal venous system [42, 43]. Both of these alternative venous routes serve as potential etiologies of varicocele recurrence following successful interruption of the internal spermatic vein [35, 42].

The remaining venous outlet after successful varicocele repair consists of the paired deferential veins that typically drain into branches of the internal iliac vessels [41]. The deferential vessels lie within an investment containing the vas deferens, which can often be visually distinguished by marked tortuosity. It is worth mentioning that the deferential artery, also a product of the internal iliacs, serves as the principle arterial supply of the testis should the internal spermatic artery be incidentally ligated. The vas deferens and its sheath lies posteriorly between the internal and external spermatic fasciae (Fig. 17.2), a useful feature for excluding the vas deferens during exposure [41, 44].

In regard to varicocelectomy, key features of the vascular anatomy include the progressive branching of both internal spermatic vein and artery. The subinguinal approach, therefore, will be characterized by increasing vascular complexity. The number of veins can often number in the tens, whereas multiple arteries serve as the rule rather than the exception [43, 45]. A firm understanding of these basic anatomical principles is necessary for proficient subinguinal microsurgical varicocelectomy, a prerequisite that is especially pertinent in difficult fields that may be subject to scar, poor visualization, or an exposure placed inadvertently too distal along the cord.

Surgical Technique

General Considerations

Prior to incision, proper instruments facilitate success (Table 17.2). Emphasis is placed on the availability of the microdoppler with a 1.2 mm tip, an instrument that will help address the multiple arteries that are expected [46]. Level I evidence now exists highlighting the utility of microdoppler assistance and should now be considered standard of care [47]. A bipolar cautery is also a necessity, providing effective hemostasis without thermal damage to nearby structures. A bipolar with a Jeweler's forcep and 0.4 mm tips are the authors' preference.

The patient is positioned supine with the arms abducted. The decision to sit or stand during the procedure is a matter of surgeon preference. Operating chairs are available that provide distal arm stabilization and chest support, which significantly improves fine motor control. Likewise, if a standing position is chosen, the surgeon should rest their wrists and hands on the patient to preferentially engage only the distal joints and musculature.

Table 17.2 Minimal necessary equipment for the subinguinal microsurgical varicocelectomy

Macro	Micro	Disposables
Operating microscope capable of 20× magnification	Fine tip bipolar hand piece	4–0 Silk ties
Microdoppler	Micro-needle holders × 2	Surgical clips
Cautery generator for both monopolar and bipolar current	Iris scissor	1" Penrose drain
Basic open kit: Small abdominal retractors × 2 Toothed and smooth Adson × 2 Babcock clamp Small needle driver Clip applier Scalpel Mosquito clamps × 4 Schnidt curved clamp Small Metzenbaum scissor	Jeweler's forceps	Vessel loops × 2
	Micro-forceps (smooth or toothed) × 2	#15 Scalpel blade
		Microdoppler probe and cord
		3–0, 4–0, and 5–0 absorbable sutures for closure

Hair is removed with surgical clippers. It is prudent to prep the patient from the umbilicus to two finger breadths below the inguinal crease should it become necessary to extend the incision to optimize exposure. The scrotum should be draped into the field to allow for intraoperative manipulation and delivery of the testis.

Initial Approach

Multiple incisions have been utilized to accomplish adequate exposure for a subinguinal approach. Our standard practice utilizes a 2–3 cm incision along Langer's lines, located above the external inguinal ring to optimize cosmesis. The size of the incision is gauged to allow for unencumbered delivery of the testis. Marking the proposed incision is most important in bilateral cases to ensure symmetry. The incision is carried down to the dermis sharply and finished with the monopolar cautery using the cutting waveform. The bipolar cautery is an effective means to address cutaneous bleeders without imparting significant thermal injury to the skin. Camper's and Scarpa's fascia are divided over a curved clamp. The external pudendal artery and vein are typically encountered at the inferior aspect of the wound and, more rarely, the superficial epigastric artery and veins may be met at the superior aspect of the wound.

Blunt dissection is then carried into the scrotum over the cord. A curved index finger hooked into the external ring, with a small abdominal retractor drawing distally from this location, will allow for expedient and clean visualization of the spermatic cord. A Babcock clamp is then used to atraumatically deliver the spermatic cord into the field, allowing for a 1" Penrose drain to be placed beneath. Using the Penrose as a manipulator, distally applied counter-tension can be applied to the cord, which allows the surgeon to finger-dissect circumferentially within the inguinal canal. This maneuver frees the cord and delivers a more proximal segment into the exposure. In the senior author's experience, when returning years later for the rare hernia repair in a post-varicocelectomy patient, the prior ligatures are typically observed in the mid-inguinal canal. Thus, the subinguinal

technique can approximate the ligation site of an inguinal approach and capitalize on a less-complex vascular anatomy [43, 45].

The spermatic cord is then released and allowed to rest on the Penrose drain. The external spermatic fascia is elevated between two forceps and carefully split with the monopolar cautery, preferably between muscle fibers. A tag suture to mark the proximal apex of this muscle splitting incision is useful for orientation and should the surgeon choose to close this layer at the conclusion of the procedure. Both the surgeon and assistant, thereafter, switch to micro-instruments and exclusively use bipolar cautery for the remainder of the cord dissection. The internal spermatic fascia is elevated in a similar fashion as the external layer utilizing micro-forceps. A fine scissor is then used to incise this layer with care to avoid underlying structures. Placement of clamps along the internal spermatic fascia provides exposure and can be positioned to isolate the vas deferens posteriorly. Again, a more proximal location along the spermatic cord can be obtained by sequentially incising and "marching" these clamps along the internal spermatic fascia towards the external ring. The final exposure consists of the free Penrose drain providing elevation to the cord and four clamps placed to maintain a square of internal spermatic fascia while excluding the vas deferens below (Fig. 17.3).

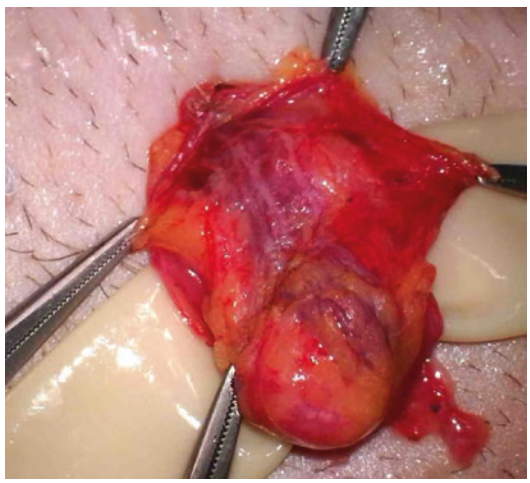

Fig. 17.3 Image demonstrating proper exposure of the spermatic cord

When reading the subsequent description below, please keep in mind that it remains at the discretion of the surgeon if proximal exposure becomes necessary. Occasionally, an extremely complex vascular anatomy is encountered in the subinguinal region, and in this scenario, the external oblique should be opened. The proximal cord should then be addressed utilizing standard microscopic techniques.

Vessel Identification and Ligation

Once the vas deferens is identified and isolated, the field is irrigated with saline to allow a coupling interface for the microdoppler. Effective use of the microdoppler probe maintains a 60 degree angle of insonation with just a thin film of irrigation solution between the doppler tip and the underlying tissue. Avoid any pressure on the doppler tip for the best effect. A crude understanding of the arterial anatomy will become evident, i.e., the rough location and number of arteries. This initial survey will help identify an early approach to minimize the risk of arterial injury.

Commonly, a few large obscuring veins will require division prior to approaching the artery. The surgeon should select one to three venous structures that are easily isolated and will optimize visualization of an area of positive doppler signal. Internal spermatic veins are invested in a thin adherent membrane that also contains a network of miniscule lymphatics. To preserve these structures, and to allow for a clean isolation of the vessel, the vein should be firmly grasped with the micro-forceps, while the tips of the micro-needle holder are pressed against the vein wall to bluntly sweep downward. A rent will be created in the surrounding membranous layer through which the vein is regrasped with the micro-forceps. A similar maneuver is repeated on either side of the vein until a tunnel beneath the vessel develops. In this fashion, a vein can be reliably separated from other adherent structures, most notably any small arteries beneath. The micro-forceps are passed through the tunnel and used to grasp two 4–0 silk ties (Fig. 17.4). Using one

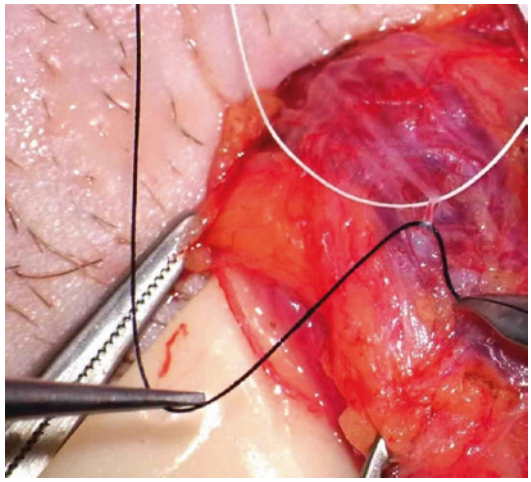

Fig. 17.4 Vein ligation with two 4–0 silk ties. In this example, an "H" pattern is observed with a branch connecting two veins. An "H" or "X" pattern of interconnected veins is usually indicative of an underlying artery

black and one white tie simplifies finding which two ends go together. The vein is ligated and divided using fine scissors. At times, one or both of the ties can be used as a handle when approaching deeper cord structures. It should also be mentioned that some surgeons prefer to clip the veins in lieu of ties. Our preference is to use silk ties near any arterial structure, with judicious use of clips for more peripheral vessels.

As the artery is approached, it is often surrounded by a dense plexus of small veins. The interconnections often form "X" and "H" patterns and are almost always indicative of an underlying artery (Fig. 17.4). The artery often appears as a small silvery-reddish structure; however, visual identification of arteries is neither sensitive nor specific. A suspected artery should never be grasped. Instead, surrounding venous structures may be handled with the micro-forceps and used to obtain tension and counter-tension against the micro-needle holders. The venous plexus should be ligated and divided until adequate exposure is obtained. A tunnel around the artery is begun by allowing the micro-needle holders to slowly spring open to spread-dissect tissue. The instrument's tips should be placed on either side of the artery with any spreading motion occurring along the axis of the vessel. Again, the surgeon may grasp surrounding venous stumps to provide cord elevation and cautious counter-tension. Adequate depth is obtained when the micro-needle holders can be convincingly passed beneath the artery. The micro-needle holder is then passed a few millimeters beyond the artery, which will invariably elevate additional tissue. With gentle elevation, the micro-needle holders are withdrawn, allowing these additional tissues to slip off the tips. The withdrawing motion is continued until only the artery remains above the instrument, which is confirmed by visualization of a clean arterial wall. The tips of the micro-needle holder must be confirmed beyond the arterial wall prior to the final spreading maneuver, which is conducted by pushing the instrument's tips firmly into one's fingertip prior to spreading.

Confirmation of the artery should then be sought by use of the microdoppler. The positive predictive value of a doppler signal is reliable, however the absence of pulsations is not. The artery may be in spasm or kinked against the internal ring, and it is commonly necessary to irrigate with papaverine to facilitate dilation. In the accompanying Video 17.1, a cleanly isolated artery can also be confirmed by gently elevating the vessel with the micro-needle holder until it blanches. Slowly dropping the instrument toward the cord will reveal an arterial blush. Note that this strategy will not work if another vessel has been isolated with the artery. A confirmed artery should then be marked by a short vessel loop, which can be grasped with the micro-needle holders (Fig. 17.5). Cutting the vessel loop tip to a taper allows easy passage under the vessel. The ends of the loop are secured together with a medium clip.

In the above stepwise fashion, the surgeon progresses through the cord until all internal spermatic veins are ligated and all arteries are surrounded with vessel loops. The plane of ligation should be consistent, as the tortuosity of these vessels can become easily confusing (i.e., the same vessel may be ligated multiple times, which adds unnecessary operative time). At the conclusion of this portion of the procedure, the surgeon should elevate the cord with the middle

finger of his nondominant hand (Fig. 17.5). The cord is then spread out and drawn over the tip of the finger to systematically examine for any remaining venous structures. At least two to three lymphatics should be spared to prevent postoperative hydrocele. These structures should have crystal clear fluid within their lumen and may be characteristically identified by a scalloping of the vessel wall (Fig. 17.6). Reinspection of the artery is warranted, as often miniscule veins may be closely adherent, a potential source of recurrence. These structures can either be peeled off the artery using the Jeweler's forceps or cauterized in place using the bipolar on low current settings.

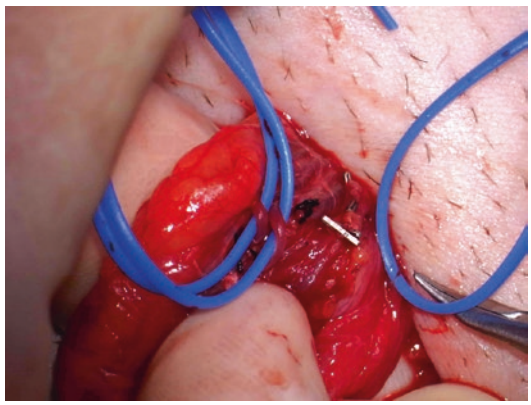

Fig. 17.5 Confirmed arteries are isolated and identified with vessel loops. In this image, the surgeon is splaying the cord over the middle finger to systematically survey for any missed venous structures. Opposing the thumb against the cord, with the middle finger providing a backing, stabilizes the cord as the surgeon thins the cord to step through each segment of tissue

Preservation of arteries adds considerable time and complexity to the case. It is notable that in other surgical techniques the artery is ligated purposefully (i.e., in laparoscopy). In animal models, ligation of the artery has been observed to decrease intratesticular testosterone and to negatively affect the Johnsen score on testis biopsy [48]. Clinically, multiple groups have observed no significant difference in semen parameters or fertility outcomes when individuals were subjected to either artery preserving or ligating laparoscopic varicocelectomy [49, 50]. The majority of these studies were conducted in adolescent patients, and we contend that the reserve and ability to establish effective arterial collateralization are greater in the pediatric population. Testicular atrophy, although rare, has been observed in the adult population following inadvertent ligation of the testicular artery [51]. Until comparable and robust studies have been conducted in the adult male, preservation of the testicular artery should be considered standard of care during subinguinal microsurgical varicocelectomy.

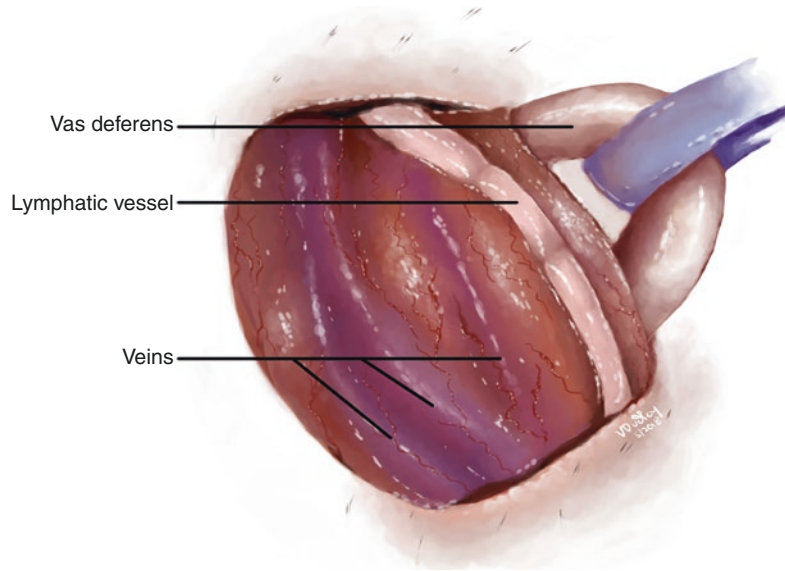

Fig. 17.6 Identification of lymphatics within the spermatic cord by the scalloping pattern

Alternative Venous Drainage of the Testis

As outlined previously, recurrence following varicocelectomy may occur due to redundant venous drainage of the testis through either external spermatic veins or via a gubernacular tract [41–43]. While running the cord over the middle finger, any cremasteric veins should be clipped and divided. Attempts should be made to identify and preserve the cremasteric artery, which can be identified via the blanching technique (see Video 17.1). We are able to identify and preserve at least one cremasteric artery in 90% of cases. The underlying Penrose drain is then elevated and the base of the wound inspected. Any external cremasteric vessels not enclosed by the Penrose should then be addressed.

Delivery of the testicle to visualize gubernacular veins remains debated [52, 53]. Early accounts established this collateral system as a rare cause of varicocele recurrence, and we argue that these vessels should be taken to optimize outcomes [35, 42, 54]. Two recent randomized control trials have found benefit in terms of recurrence rates when the gubernacular veins were ligated, although an earlier trial found no clinically significant advantage [55–57]. In our practice, we continue to deliver the testis. Even if gubernacular collaterals are not contributing to the varicocele, and they are simply "vents" for the increased venous pressure that occurs after ligating the internal and external spermatic veins, ligating these vents further increases the venous pressure, and when rerunning the cord, we have been shocked at how often previously undetected internal or external spermatic veins have enlarged and become visible. We are convinced that rerunning the cord after ligation of the gubernacular veins has significantly reduced our failure rate.

Upon delivery of the testis, the gubernaculum can be bluntly thinned to improve visualization (Fig. 17.7). In a similar fashion to screening the spermatic cord, the middle finger of the nondominant hand is placed under the gubernaculum and provides elevation. The tissue is then systematically drawn over the finger using the aid of the micro-needle holder. Any encountered vascular structure entering the tunica vaginalis is clipped and divided. Should a small amount of hydrocele fluid be identified, a window can be optionally created in the tunica vaginalis with hemostasis obtained by the monopolar cautery. Occasionally, a formal hydrocelectomy is warranted.

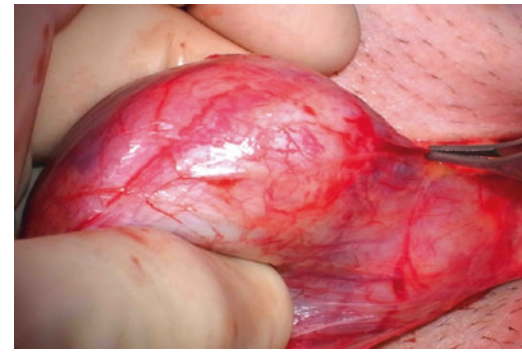

Fig. 17.7 Delivery of the testis with blunt dissection to thin the gubernaculum. Only vessels passing between the gubernaculum and the tunica vaginalis require intervention

Final Assessment and Closing

Adequate hemostasis must be ensured prior to returning the testis to the scrotum. At the conclusion of the ligation, a strong impulse should be palpable distal to the plane of dissection when the spermatic cord is squeezed above the testis. A lack of impulse should prompt another screen of the cord until the surgeon is convinced that every venous structure except for the vasal veins has been interrupted. The impulse maneuver also provokes sites of bleeding that need to be addressed with the bipolar cautery or further ties.

Although optional, we prefer to close the external spermatic fascia to reestablish the anatomic planes and to cover the exposed testicular arteries. This closure should be accomplished loosely with 2–3 interrupted 5–0 absorbable sutures. Subsequent steps include closure of the Scarpa's and Camper's fasciae. The skin is typically reapproximated with deep dermal interrupted sutures, followed by a running subcuticular stitch reinforced with steristrips.

Postoperative Counseling

As is common with all varicocele repair techniques, the subinguinal microsurgical varicocelectomy is an outpatient procedure. We advise our patients to ice the scrotum for 48 hours following the surgery and to wear an appropriately sized scrotal supporter. The use of perioperative celecoxib has been found useful in similar procedures [58]. Narcotic utilization following varicocelectomy is highly variable, but does not typically pass post-op day three or four.

Patients may return to desk work in two to 3 days with cautious activity. As the inguinal canal remains intact, there is no increased risk for hernia. Heavy lifting should still be avoided as the resulting intra-abdominal pressure will tend to stress venous hemostasis in the scrotum. We evaluate our patients 4 weeks post-op following the procedure to ensure proper healing of the wound and at 3 and 6 months to evaluate for failure. When the varicocelectomy fails, this is almost always detectable at 4 weeks by a persistent impulse on Valsalva and persistent veins that collapse when supine. After repair of large varicoceles, thrombosed veins may take up to 3 months to resolve, and there should be no impulse on Valsalva and no change in the cord from the upright to the supine position. A repeat semen analysis can be obtained at 3 and 6 months to evaluate for improvement [59]. This interval is also adequate to evaluate for testicular atrophy.

Brief Comments Regarding the Recurrent Varicocele

Varicocele recurrence may be confirmed by either physical exam or ultrasound. Due to post-op scarring, it is important to note that the sensitivity of the physical exam may be substantially decreased. It only requires one patent vein to eventually cause filling and dilation of all venous structures distal to any ligated segments. It is impossible to perfectly dissect the ligature plane from an earlier varicocelectomy, and so it is difficult to accurately identify which veins were previously ligated when encountering these dilated vessels through a new surgical approach. Therefore, it is rare to identify the solitary vein that was missed initially. Although external spermatic and gubernacular veins were extensively discussed, the vast majority of varicocele recurrences occur due to a missed internal spermatic vein [60]. These represent technical errors.

A number of options exist in addressing the recurrent varicocele. A general rule is to approach the spermatic cord with a different exposure to avoid the site of maximum scar tissue. Amelar preferred the use of embolization for the recurrent varicocele, a strategy that benefits from concurrent venography to define the patent vasculature [61]. In the senior author's experience, the subinguinal approach often remains viable despite a previous subinguinal microsurgical varicocelectomy. The safety of this approach has been established by a small series by Grober et al. [62] The prior plane of dissection is easily identified by the presence of clips and surgical ties. A site just proximal or distal to the prior ligatures will often facilitate effective repair. Should unfavorable conditions prevail, the external oblique can be opened to facilitate a proximal approach. Finally, the gubernacular and external spermatic veins must be addressed during a procedure for the recurrent varicocele.

The Role of Microsurgical Training—A Call for Further Study

The subinguinal microsurgical varicocelectomy requires the development of a complex and demanding set of skills. The tissue handling techniques required for successful repair cannot be gleaned from a review of this type. In other areas of urology, objective measures are now being developed to assess trainee proficiency and to define a learning curve to judge competency. For instance, studies such as Abboudi et al. have begun to define the minimal case experience required to perform a robotic-assisted laparoscopic prostatectomy [63]. Along similar lines, the benefits of simulation have now been clearly delineated for techniques such as laparoscopy [64].

In our center, we utilize a microsurgical training lab to teach suture handling and tying under the operating microscope [65, 66]. We have observed a minimal requirement of 500 microsurgical knots to obtain an appropriate level of proficiency for progression to an animal model. Other centers have also attempted to develop a systematic method for microsurgical training [67–69]. Continued work is required to mature this field and to ensure the optimal and standardized training of future urologists.

Conclusions

Varicocelectomy provides an effective treatment for male infertility and androgen deficiency in the appropriately selected patient. Multiple options exist to achieve repair, and the ultimate selection hinges on surgeon experience, available resources, and patient characteristics. The best evidence supports subinguinal microsurgical varicocelectomy as the gold standard. The technical steps of the subinguinal microsurgical varicocelectomy rely upon appropriate exposure of the testicular artery, a systematic approach to vein ligation, and preservation of the vas deferens and lymphatics. The majority of early and late failures are due to missed internal spermatic veins. To avoid this technical error, we recommend multiple passes through the spermatic cord to inspect for missed veins. These structures will dilate over the course of the operation, which will facilitate identification and ligation. Finally, considerations for approaching the recurrent varix are outlined. These procedures can be challenging due to postsurgical scarring with subsequent loss of anatomic planes. All internal spermatic veins must be ligated again, and alternative routes of venous return (gubernacular and external spermatic veins) must be addressed. It should be stressed that, despite the detail of this review, our remarks cannot substitute for an appropriate and extensive foundation of microsurgical training.

Review Criteria

A systematic review was conducted using PubMed and Google Scholar. Search dates were restricted to January 1950 through May 2018. Study identification was conducted using the following search criteria: "varicocele", "varicocelectomy", "male infertility", "varicocele recurrence", "varicocele embolization", "microsurgical", "subclinical", "semen parameters", "pregnancy rates", "reactive oxygen species", "DNA fragmentation", "azoospermia", "oligospermia", "scrotal hyperthermia", "venous reflux", "post-operative pain", "hydrocele", "surgical training", "gubernacular", "external cremasteric", "venography", "testicular hypotrophy", "ultrasound", "varicocele grading". Only literature published in the English language was reviewed.

Multiple Choice Questions and Answers

1. When unusually complex vascular anatomy is encountered during a subinguinal exposure, an accepted surgical strategy is:
   (a) Spermatic cord-freeing techniques to gain more proximal access
   (b) Extension of the skin incision and committing to an inguinal approach
   (c) Deferring particularly difficult or small veins, especially when adherent to the artery, until after testis delivery to allow time for venous dilation
   (d) **All of the above**
2. Proper use of the microdoppler includes all of the following except:
   (a) Ensuring a proper angle of insonation
   (b) Utilizing known arteries as an intraoperative control to rule out equipment malfunction
   (c) Minimization of tissue compression
   (d) **The presence or absence of a pulsating waveform is reliably indicative of an artery and vein, respectively**

3. The following are effective strategies of addressing the recurrent varicocele except:
   (a) The region of maximal scar formation may be avoided by choosing an alternative exposure than that of the original repair
   (b) **The subinguinal approach should be avoided for the failed subinguinal microsurgical varicocelectomy**
   (c) The surgeon may choose to dissect the spermatic cord proximally or distally to that of the original repair
   (d) Delivery of the testis with ligation of the gubernacular veins is mandatory
4. The most common cause of varicocelectomy failure is:
   (a) **Dilation of unidentified internal spermatic veins**
   (b) Patent gubernacular veins
   (c) Missed external cremasteric veins
   (d) Proximal collateralization of the right and left venous systems
5. Regarding the diagnosis of a varicocele, ultrasound is a useful adjunct to the physical exam when:
   (a) There is suspected varicocele recurrence following a repair
   (b) Prior scrotal or inguinal surgery precludes adequate palpation of the spermatic cord
   (c) The testis is high-riding or retractile
   (d) **All of the above**

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18 Laparoscopic Techniques for Varicocele Repair

Roberto Mendez-Gallart
and Maria Garcia-Palacios

Key Points

- Varicocele surgery has demonstrated to be effective in the improvement of male infertility, semen quality, and pregnancy rates. The goal of surgery for varicocele in adolescents and young adults is to improve the potential for future fertility.
- The reviewed data suggest that laparoscopic varicocele ligation is therapeutically superior to open surgical and embolization/sclerotherapy procedures. Laparoscopic varicocelectomy appears to reduce postoperative morbidity.
- Several laparoscopic procedures have been described for treating varicocele: however, approach to laparoscopic varicocelectomy should be based on the physician's experience and the surgical options available.
- The major disadvantage of laparoscopic varicocelectomy is the high rate of hydrocele formation, but this worrisome complication may be reduced by using a lymphatic sparing procedure.

Introduction

Varicocele is the most common correctable etiology found in adults with infertility and has been implicated as a cause in 35–50% of men with primary infertility [1, 2]. The incidence increases progressively from the age of 10 years and can be found in approximately 15% of all adolescent males and young adults [3]. The condition can often lead to testicular atrophy and subsequent infertility in young patients [4].

Surgical treatment of varicocele in adults should be considered when this condition is clearly palpable on physical examination and the semen parameters or sperm function tests are abnormal [5–13]. Presently, the data suggest that subclinical varicocele repair for male factor infer-

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R. Mendez-Gallart (✉)
Pediatric Surgery & Urology, University Hospital of Santiago, Santiago de Compostela, A Coruña, Spain
e-mail: roberto.mendez.gallart@sergas.es

M. Garcia-Palacios
Pediatric Surgery & Urology, University Hospital of Santiago, Santiago, Galicia, Spain

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tility is not beneficial. In adolescent varicocele, indications of surgery must be individualized based on clinical data, including pain, testicular asymmetry, endocrine parameters, and abnormal color Doppler findings [14]. The goal of surgery for varicocele in children and adolescents is to improve the potential for future fertility [15, 16].

Regardless of the surgical procedure used for correction, varicocele repair results in testicular growth, increased serum testosterone, and significant improvement in semen parameters, including sperm concentration, motility, and sperm morphology [17–19].

Diverse surgical techniques have been described to correct varicocele, but there is not currently a gold standard for its treatment. It is evident from the reviewed literature that each procedure has its own set of advantages and disadvantages [20–22]. Surgical options for treating varicocele include retroperitoneal ligation of the testicular vessels above the internal inguinal ring (Palomo procedure); retroperitoneal ligation of the vein alone sparing the artery (Bernardi technique); ligation of the spermatic veins close to the inguinal canal (Ivanissevich procedure); microscopic sparing of the arteries and lymphatics while cutting the spermatic veins within the inguinal canal (inguinal microsurgical procedure); laparoscopic high ligation of the spermatic vessels (retroperitoneoscopic varicocelectomy); antegrade scrotal sclerotherapy of the veins (Tauber technique); and percutaneous retrograde sclerotherapy. All of this variety of surgical techniques involves the ligation of the spermatic veins. Main differences are related to the surgical approach to the vessels, the level of the ligation, and whether the artery and/or lymphatics are spared or ligated along with the veins.

The ideal surgical procedure would be the one that has the lowest recurrence and complication rates, but when comparing these techniques of varicocele treatment, the reviewed literature is not conclusive about the results.

Surgical Laparoscopic Procedures

Laparoscopic varicocelectomy is quite similar to the retroperitoneal high approach (Palomo procedure): ligation of the spermatic vessels occurs at the same level above the internal inguinal ring [23] (Fig. 18.1). Advantages to laparoscopic surgery are the small incisions, minimally invasive surgery, optical magnification, and a fast recovery, reducing postoperative morbidity. The global success rate of the laparoscopic approach has been reported to be even better than the Palomo procedure, with only 1% failure rate and minimal complications [24–26]. Diverse laparoscopic procedures have been used to correct varicocele, although some of these techniques are only refinements to the standard approach.

Conventional Laparoscopic Approach

Conventional laparoscopic varicocelectomy is performed under general anesthesia and the use of three ports, one 10-mm/5-mm for operative telescope and two further 5 mm ports for scissors, clip applied, and grasper. The patient is placed supine on the operating table in the Trendelemburg position, and a Foley catheter is introduced to empty the bladder (removed at the end of the procedure). A Veress needle for CO_2 gas inflation may be used before the first trocar placement, but the blunt open or Hasson's access to the peritoneum through an infraumbilical 1-cm incision is usually performed. A 5-mm/10-mm trocar is introduced into the peritoneal cavity and a 5 or 10-mm 30° operative telescope is inserted through it. Limited CO_2 gas inflation pressure of 15 mmHg is recommended. The surgeon stands on the patient's right side and the assistant opposite the surgeon. Two additional trocars are required: one 5-mm in the right lower abdomen and the other in either the midline or the opposite lower quadrant for passage of the clip applier. After a pneumoperitoneum is well-established, the internal inguinal ring and the testicular vessels are identified. Peritoneum over these spermatic vessels is gently incised using dissecting shears two centimeters above the internal ring. By using a fine-tipped dissector, surgeon can dissect the artery free from the veins. Once the testicular veins are identified, they can be ligated with endoclips and divided. Alternatively, the gonadal vessels may be ligated "en bloc" without sparing the artery. Reperitonealization of the small window is not necessary. Before any instruments are removed, the

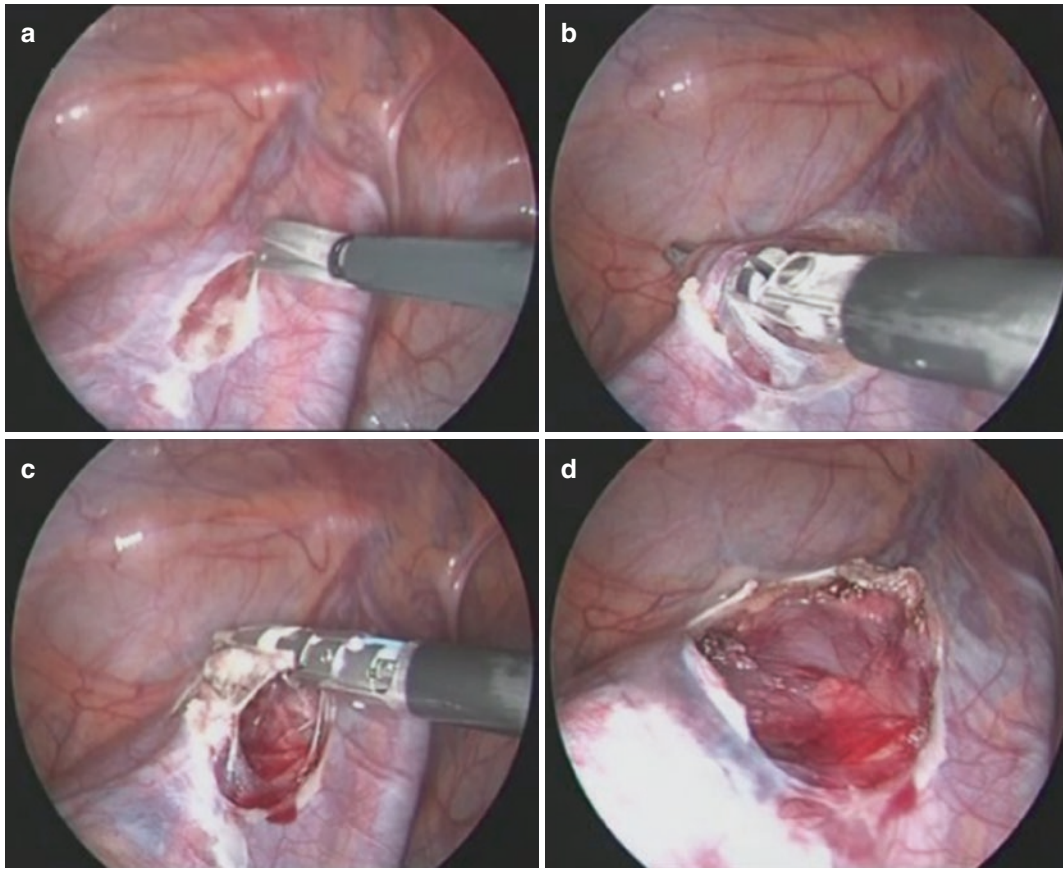

Fig. 18.1 (**a**) Peritoneal window is made at the level of spermatic dilated veins using endo-scissors. (**b**) By using the curved endo-dissector, vessels are liberated from the retroperitoneal connective tissue and the psoas muscle. (**c**) Ligasure™ sealant device is applied two to four times to ensure ligation. (**d**) Peritoneal gap remains open

pneumoperitoneum must be lowered to discard any bleeding from the surgical bed. Ports are removed under direct vision to ensure that there is no hemorrhage from the trocar sites. Each incision is infiltrated with bupivacaine 0.25%. For closing the wounds, 4-0 absorbable sutures in the subcutaneous tissue and skin are recommended, placing steristrips over the incisions. Intravenous acetaminophen is the only analgesic therapy routinely used for pain management. Procedure can be done on a day-case surgery basis [27].

Two Trocar Laparoscopic Procedure

The first 5-mm optical umbilical port is inserted as in the conventional laparoscopic approach. Only one additional 5-mm working port in the right lower quadrant is necessary. A peritoneal window is done with dissecting shears at the level of spermatic dilated veins before entering the internal inguinal ring (a few centimeters above). The scrotum is usually compressed for the filling of the spermatic veins. Dissection of the adventitial tissue surrounding the testicular vessels should be kept to a minimum to avoid disruption of lymphatics. No attempts are made to locate and preserve the spermatic artery. By using the blunt-tipped dissector, vessels are liberated from the retroperitoneal connective tissue and the psoas muscle. Veins must be ligated "en bloc" and divided using the Ligasure® (Tyco Healthcare) vascular sealing device (5-mm in diameter). The Ligasure® sealant is applied two to four times to ensure coagulation. The peritoneal window is left without reperitonealization

for healing without closure (Fig. 18.1). The surgical area must be inspected for hemostasis. A valve of the umbilical port is left open to deflate the abdomen. At the end of the procedure, the two skin wounds are infiltrated with bupivacaine and closed with 4-0 absorbable sutures [28, 29].

Retroperitoneoscopic Approach

Retroperitoneoscopy offers a versatile access for many urologic indications such as varicocele. The procedure is done under general anesthesia. The patient is positioned in a right lateral decubitus position, with a roll underneath the lumbar region and a bend in the table to widen the space between the 12th rib and the iliac crest. The surgeon stands on the patient's right side (behind) and the monitor opposite the surgeon. A transverse incision of 1 cm is made below the apex of the 12th rib for the introduction of a 5- or 10-mm, 0° operating telescope. A muscle-splitting blunt dissection is used to gain access into the retroperitoneal space. A ballooned trocar is introduced into the retroperitoneal space under direct vision. CO_2 gas inflation to 12–15 mmHg is reached to induced retropneumoperitoneum. Moving the tip of the telescope to free retroperitoneal fibrous tissues progressively enlarges the working window created by the carbon dioxide. The psoas muscle, ureter, and testicular vessels must be clearly identified. Spermatic vessels are closely attached to the posterior part of the peritoneum. The artery and the veins must be gently dissected off the peritoneum, coagulated using bipolar electrocautery or endoclips, and finally divided. Incisions are closed and infiltrated with bupivacaine. Patients can be discharged the same day of the surgery [30–32].

Single Incision Laparoscopic Surgery (SILS)

Single incision laparoscopic surgery (SILS), since its first description in 2007, has been proven to be feasible and effective. SILS for varicocelectomy has been reported to be a safe and effective alternative to conventional laparoscopic varicocelectomy [33–36]. Moreover, this procedure is especially effective in bilateral cases. The patient is placed in a standard supine position under general anesthesia. An umbilical transverse 2-cm incision is performed. The underlying fascia is incised vertically and the access to peritoneal cavity is secured under direct vision. The flexible SILS port is inserted into the abdomen. Once the three ports (one 10-mm and two 5-mm) provided with the SILS are introduced, a pneumoperitoneum is inflated to 15 mmHg. A 10-mm optical 30° telescope is then inserted and the patient is placed in Trendelemburg position. A Grasper and dissecting scissors are inserted through the 5-mm working ports. A peritoneal gap is obtained to expose the gonadal vessels. Gently grasping and elevating the vessels allows separation of the artery and lymphatics from the veins. The isolated veins are then clipped (or sealed with bipolar electrocautery) and divided. At the end of the procedure, the SILS port is removed and the fascia is closed using 3-0 absorbable sutures followed by umbilical wound closure.

Robot-Assisted Varicocelectomy

Few authors have reported their initial experience with robot-assisted varicocelectomy during last years. While the cost associated with a surgical robot is certainly a significant limiting factor for the widespread use of robotic-assisted varicocelectomy, there seems to be clear benefits of this approach compared with the conventional laparoscopic varicocelectomy. Advantages of the robotic approach include 3-D optics to allow improved precision of dissection, enhanced stability, and ergonomics of instrument handling for surgeons to overcome the limited mobility imposed by the use of straight laparoscopic instruments and increased degree of freedom in the range and extent of instrument manipulation. Undoubtedly, with the improvement and accessibility of surgical robots and enhancement of surgeon's skills, robot-assisted laparoscopic varicocelectomy will find its place in the therapeutic armamentarium for treating varicocele [37, 38].

Artery/Lymphatic Sparing Procedures

Lymphatic Sparing

The laparoscopic Palomo procedure of high ligation of the testicular vessels offers a low recurrence rate, but with an increased risk of reactive hydrocele (up to 30% with long-term follow-up) [39]. The underlying cause of this complication may be due to the interruption of the lymphatic return as a consequence of "en bloc" ligation of the spermatic vessels including the lymphatics.

Some authors affirm that lymphatic preservation may be relevant not only to prevent reactive hydrocele but also avoid the impairment of testicular function [40]. The lymphatic sparing procedure may be used with all the surgical techniques routinely employed for treating varicocele. Adequate visualization of lymphatics is not straightforward. In order to reliably and objectively identify and spare the spermatic lymphatic vessels surrounding the testicular veins, a dye-assisted laparoscopic procedure has been recommended. Different dyes are used regularly to stain the lymphatics: subdartos scrotal injection of isosulfan blue dye [41, 42]; injection of methylene blue under the tunica albuginea [43]; and injection of Indigo carmine [44]. However, the use of dye agents is not free of complications; methylene blue dye can cause local complications such as skin and fat necrosis and isosulfan blue has been associated with urticaria, generalized rash, pruritus, hypotensive reactions, and even anaphylaxis [42, 45].

Independent of the dye used for lymphatic sparing, all authors agree that this is a valuable refinement in reducing the incidence of reactive hydrocele after laparoscopic varicocelectomy, with similar recurrence and catch-up growth rates as non-sparing patients [46]. Diverse studies have reported nearly zero rates of postoperative hydrocele with the dye-assisted laparoscopic approach [45].

Artery Sparring

Preservation of the testicular artery during laparoscopic varicocelectomy still remains controversial. Some authors support the necessity of sparing the artery from the spermatic veins in the belief that artery ligation may jeopardize (impair) testicular development and semen parameters, although most long-term studies have not found significant differences in catch-up growth and no instance of testicular atrophy [47]. Definitely, what has been clearly established by diverse authors is the high rate of persistent/recurrence of varicocele in artery-sparing patients [48, 49].

Considering the benefits of less persistence/recurrence rate after surgery, the shorter operating time, and similar results with respect to testicular growth, artery non-preserving approach may be preferable to artery sparing in laparoscopic varicocelectomy. However, comparative multicenter studies are necessary to definitely advocate for preservation or ligation of the testicular artery during laparoscopic varicocele surgery [50].

Outcomes and Complications

One of the most controversial facts in the treatment of varicocele is to determine the ideal surgical procedure. Overall, surgical treatment successfully eliminates over 90% of varicocele regardless of the procedure used. The reported data suggest that laparoscopic varicocelectomy is a rapid, safe, effective, and minimally invasive option therapeutically superior to open surgical and embolization techniques. Furthermore, laparoscopic approach appears to reduce postoperative morbidity [51–55]. Moreover, other authors conclude that there is a cost benefit with laparoscopic procedure related to open surgery, presumably due to the shorter operating times and postoperative complications requiring emergent management in patients undergoing subinguinal microscopic varicocelectomy [56].

Pastuszak et al. [57] reported that the preferred surgical techniques for pediatric urologists were laparoscopic (38%), subinguinal microsurgical (28%), inguinal (14%), and open Palomo (13%). The authors remark that management of pediatric varicocele appears to have remained stable over the past decade, with a light increasing

use of the laparoscopic approach [57]. Diverse authors have shown that laparoscopic varicocelectomy is the most commonly used approach in adolescent patients [58].

Nonsurgical procedures like embolization may seem appealing initially, but it has several disadvantages: it is a complex invasive procedure that may take 1–3 h to complete and, in 15%, may not be possible to accomplish due to technical reasons. Moreover, some problems as radiological skills, sedation, risk of venous perforation, and migrations of the device make its use controversial [59].

However, hydrocele formation has been related as the most common complication after laparoscopic treatment with rates ranging from 5% to 39%. When present, approximately 50% of hydroceles will develop to a size that produces discomfort and warrants surgical correction [60, 61].

Recurrences after varicocele repair vary from 0% to 35%, depending basically on the surgical technique employed for correction [62]. The persistence/recurrence rate of laparoscopic varicocelectomy is in the range of 6–15% [63]. Recurrences are supposed to be caused by collateral veins of the periarterial plexus unnoticed during surgery. Magnification refinements may allow better visualization of these vessels, contributing to decrease the recurrence rate in the future. Laparoscopy may be useful for redo surgery in cases of recurrence regardless of the technique previously used, with reported success rate of 100% [64].

Testicular artery ligation is a common complication of diverse surgical varicocelectomy procedures, although it's inherent in laparoscopy technique. Testicular atrophy has not been reported in series of Palomo's procedure. Saving the artery is known to be associated with a higher recurrence rate; therefore, most authors routinely recommend a mass ligation of all of the vessels, including the artery [65, 66].

Other related complications of laparoscopic varicocelectomy are reported to be of less than 10% and include air embolism, inadvertent arterial rupture, genitofemoral nerve injury, intestinal injury, and peritonitis [67, 68].

Conclusions

Literature review confirms the efficacy, safety, and excellent success rate of the laparoscopic procedure to correct varicocele, especially in adolescent patients. This technique should be integrated into the laparoscopic training program for urology residents. Hydrocele formation after varicocele surgery is still a worrisome unresolved problem. Dye-assisted lymphatic sparing during laparoscopic varicocele may be useful for reducing the incidence of postoperative hydrocele. Advances in minimally invasive surgery continue to evolve and allow laparoscopic Palomo to be performed safely and rapidly as an outpatient procedure. We strongly recommend laparoscopy surgery as the gold standard in varicocele treatment, especially in pediatric, adolescents, and bilateral cases.

Review Criteria

An extensive search of studies dealing with surgical treatment of varicocele was performed using search engines such as PubMed, MEDLINE, ScienceDirect, Embase, OVID, Google Scholar, Cochrane Library, and Scopus. The end date for these searches was April 2018. The overall strategy for study identification and data extraction was based on the following key words: "varicocele", "surgery", "laparoscopy", "Palomo procedure", "varicocelectomy", and "outcomes". Articles published in languages other than English were also considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included. Book-chapter citations provide conceptual content only.

Multiple Choice Questions and Answers

Five questions with four possible choices and one correct answer

1. What are the indications for surgical treatment of varicocele?
   (a) Testicular atrophy
   (b) Repeated abnormal semen parameters
   (c) Large varicocele
   (d) **All of the above**
2. In lymphatic non-sparing procedure, the incidence of postoperative hydrocele is
   (a) Near zero
   (b) **Up to 30%**
   (c) 60%
   (d) 90%
3. The most relevant surgical procedure for correction of varicocele in children and adolescents is
   (a) Ivanissevich procedure
   (b) Bernardi technique
   (c) Percutaneous retrograde sclerotherapy
   (d) **Laparoscopic varicocelectomy**
4. The success rate of varicocele surgery is approximately
   (a) 60%
   (b) 40%
   (c) 70%
   (d) **90%**
5. When comparing the surgical treatment of varicocele with high ligation procedure (Palomo) versus suprainguinal lymphatic sparring technique, the following sentence is false:
   (a) More hydrocele incidence in Palomo varicocelectomy repair
   (b) Similar testicular atrophy in both procedures
   (c) **More testicular atrophy in Palomo varicocelectomy repair**
   (d) All of the above

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Interventional Radiology for Varicocele Treatment

19

Walid M. Mubarak, Jehan AlRayahi, and Ashley James Robinson

Key Points

- Collaboration between the urologist and interventional radiologist is of utmost importance. This includes setting realistic goals, addressing limitations of interventional and surgical techniques, and management of complications.
- When possible, seeing the patient in a multi-disciplinary clinic is favored.
- Admission, if necessary, is advised in a specialized urology ward, with a team that has in-depth knowledge of IR practice.
- Always assess sperm count before the procedure, as this provides you and the patient with a baseline and may become of medicolegal importance in case of complications.
- For the purpose of uniformity, comparison of results, and conquering learning curves, try to standardize your technique and choice of embolic agent or device.

Introduction

Varicoceles are defined as abnormal dilatation of the pampiniform venous plexus. In adults, they occur in 15–17% of men. In the pediatric population, the incidence is approximately 1% in young boys and 15% in older boys aged 14–18 years [1].

Ninety percent of varicoceles are left-sided and unilateral. Ten percent are bilateral. Right-sided varicoceles are rare and occur in less than 1% of the general population. These isolated right-sided varicoceles require thorough investigation as they may be caused by abdominal masses.

Clinical and Sonographic Diagnosis

Adult patients usually seek medical treatment for infertility. Pediatric patients usually complain of scrotal pain, which may be exacerbated by long periods of standing. The diagnosis is made by examination and can be confirmed by sonography. Both methods have a grading system. The clinical and sonographic grading systems used in our institution are as described below (see Table 19.1).

The value of ultrasound is in ruling out abdominal masses, assessing testicular vascularity, and measuring the size of the varicocele. It is strongly advised that the urologists and radiologists in the same institution use the same classification, as this facilitates management and communication.

W. M. Mubarak (✉)
Diagnostic Imaging – Interventional Radiology, Sidra Medicine, Doha, Qatar
e-mail: wmubarak@sidra.org

J. AlRayahi
Diagnostic Imaging, Sidra Medicine, Doha, Qatar

A. J. Robinson
Diagnostic Imaging, Sidra Medicine/Weill-Cornell Medical College, Doha, Ad Dawhah, Qatar

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Table 19.1 Table depicting the two major clinical and sonographic grading systems for varicoceles

Clinical grading system (Dubin et al. [2])	Sonographic grading system (Sartechi et al. [3])
1. *Grade 0* – Not palpable	*Grade 1* – The absence of varicose veins, but venous reflux with Valsalva
2. *Grade 1* – Palpable with the patient standing and performing the Valsalva maneuver	*Grade 2* – The presence of varicose veins >3 mm in diameter with the presence of venous reflux during a Valsalva maneuver
3. *Grade 2* – a moderate varicocele palpable without the Valsalva maneuver	*Grade 3* – The presence of varicose veins >3 mm with the presence of venous reflux without Valsalva maneuver
4. *Grade3* – a large varicocele that is visible without the need for palpation	

Anatomy and Classification

The anatomy has been discussed in detail in previous chapters. These are a few relevant points:

- Classic anatomy (80%):
  - The left internal spermatic vein (ISV) joins the left renal vein.
  - The pampiniform plexus of veins forms at the level of the femoral head.
  - The right ISV joins directly into the inferior vena cava (IVC).
- Normal variants [4–6] (20%):
  - The right ISV can drain into the right renal vein in 8%
  - The right ISV can drain into multiple terminating veins in the IVC and the renal vein in 16%.
  - The left ISV can drain into multiple terminating veins in the left renal vein in 20% of cases.
  - Rarely, one of those multiple terminating veins may terminate in the infrarenal IVC.
  - Multiple collateral communications exist between the retroperitoneal, peritoneal, adrenal, and portal veins.
  - The left and right ISVs at the level of L3 may communicate. This may be evident on venography.
  - A circum-aortic left renal vein may be seen in 9% of patients.
  - A retro-aortic left renal vein may be seen in 2% of patients.
  - A left inferior vena cava may be seen in 0.2–0.5% and a double inferior vena cava may be seen in 0.2–3%.

The Bähren Classification

Bähren et al. [7] developed the following anatomic classification system. This classification is probably the most well-known and widely used. Other classifications are rarely used and a literature search does not show any significant alternative for venographic/angiographic classifications.

- Type 0 – No venous reflux on venography
- Type I – Reflux into a single non-duplicated gonadal vein
- Type II – Reflux into non-duplicated gonadal vein that communicates with either an accessory gonadal, lumbar or iliac vein, or the inferior vena cava
- Type III – Reflux into duplicated gonadal vein (caudal) joining into a single trunk at the renal vein junction
- Type IV – Competent valves at the renal-ISV junction without reflux into a renal hilar/capsular collateral vessel
- Type V – Reflux into a gonadal vein with drainage into a circum-aortic renal vein

We find that the type of variation has little or no impact on technique as it follows the basic concepts of embolotherapy. The classification is probably interesting for academic purposes rather than practical purposes.

Indications for Treatment

Indications for treatment have been extensively discussed in other chapters. The main indications for interventional treatment are pain [8], infertility, and recurrence after surgical ligation. This recurrence is usually attributed to collateral circulation that was missed during surgery. Varicocele treatment is never an emergency and

pre-procedural workup and planning are paramount to procedure success.

There are no contraindications to varicocele embolization other than allergy to one or more of the agents used during the procedure.

Embolotherapy—Different Embolic Agents; Opportunities and Challenges

Many embolic agents have been used to perform varicocele embolization [4, 6, 9–15]. The most frequently used agents are sodium tetradecyl sulfate (STS) foam and coils, followed by glue. Less commonly used agents are detachable balloons, particles, and hot contrast material. We find these less commonly used agents less practical and more cumbersome to use. We discuss some of the options below.

Sodium Tetradecyl Sulfate (STS)

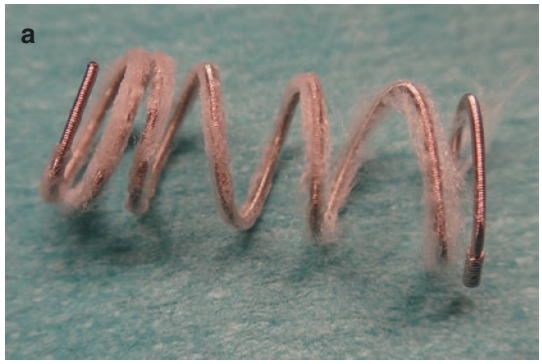
This is a commonly used sclerosant available in solution form in a concentration of 1% and 3%. It can be frothed up to create a foam for a superior coating effect and volume occupation. STS causes severe inflammation of the endothelial surface that promotes adherence of the venous walls and a reduction in the venous capacity. It is a relatively safe drug with a rare nontarget sclerotherapy effect or venous perforation or systemic leakage.

Coils

Coils are an excellent means of arterial and venous embolization. They come in a huge variety of shapes and sizes and are made of different materials. Discussing the full range and options for coil use are beyond the scope of this chapter. Their mechanism of action is generally irritation of the endothelium and promotion of thrombosis. Coils can come in detachable and nondetachable forms, with the detachable form being more expensive, but has a more controlled deployment. If the coil deployment is unsatisfactory, it may be retracted into the catheter and then redeployed. Some authors have reported good results with using coils without a sclerosant [16], with recurrence as low as 4% [17]. However, we believe this can be reduced by combining coils with sclerosants for a synergistic effect. The main disadvantage of using coils is that they may hinder reaccess into the same blood vessels in case of recurrence and re-intervention. This agent has a reasonable learning curve.

Vascular Plugs

Vascular plugs are relatively easier to deploy when compared to coils and need a shorter deployment distance. However, they are usually more expensive. They have the same mechanism of action as coils, but rely more on lumen occlusion and flow stasis to promote thrombosis. Plugs are relatively easier to deploy than coils (Fig. 19.1a, b).



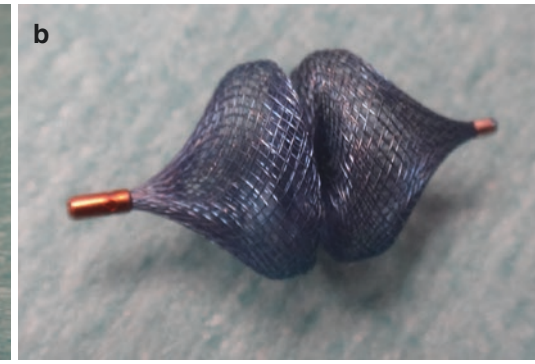


Fig. 19.1 8 mm × 14 cm Nester coil. (**a**) Photograph depicting a Nester coil (Cook Medical, USA). The tiny threads incorporated into the design purportedly aid with thrombosis in the embolized vessel. (**b**) Close-up photograph of an AVP4 Amplatzer plug usually used in low-profile vessels

Glue

Glue (*n*-butyl cyanoacrylate) is an adhesive agent. It is regarded as a form of sterile 'super-glue'. It is cheap and available and can be diluted down to different concentrations with contrast or lipiodol. Many authors have used glue as the sole sclerosant [18, 19]. It usually offers a very low recurrence rate [20]. The main disadvantage of using glue is the steep learning curve and adherence to the catheter used for injection. It is commonly used with a D10 'sandwich' technique, which means a column of Dextrose 10% is used before and after the glue injection to prevent the catheter being used from sticking to the glue and for preserving the catheter lumen. This can be difficult in varicocele embolization due to the small volumes of the veins being injected. The use of glue probably has the steepest learning curve as mastering the use of different glue dilutions can be challenging.

Absolute Ethanol

Absolute ethanol (alcohol) is a permanent embolic agent that causes rapid, irreversible, and severe denaturation of blood proteins and endothelium causing vascular thrombosis.

Percutaneous Embolization of Varicocele

The IR (Interventional Radiology) Clinic Visit

In our practice, varicocele treatment is primarily performed by urologists and interventional treatment is mainly reserved for recurrence after surgery, patients refusing surgical treatment, or patients unfit for anesthesia.

Referral to the IR clinic should be performed for any patient undergoing an elective image-guided procedure. It allows the interventional radiologist to establish rapport with the patient, request any necessary pre-procedure workup, and address any concerns or queries.

Patients can be treated on an outpatient basis under local anesthesia alone, sedation or general anesthesia. If patients opt for a general anesthetic, they may require a separate anesthesia clinic visit.

Routine pre-procedural blood workup may include a complete blood count, coagulation profile, and a semen analysis.

A plan for discharge on the day of the procedure or overnight stay in a urology ward should be discussed and a clear review of expectations and outcomes should be laid out. If the procedure is planned solely for treatment of infertility, a discussion with the infertility specialist involved may be of benefit to ensure that the procedure is in fact necessary and that a multi-disciplinary discussion has taken place. The chances of recurrence after embolization should also be approached.

Technical Considerations

Appropriate radiation protection during varicocele embolization is paramount due to the proximity to the testicles, which are radio-sensitive organs. This is even more important in children.

Regardless of the technique or sclerosants, the ultimate goal remains the same; sclerosing the ISV to decrease the blood flow to the pampiniform plexus of veins. A common and simple way to perform embolization is to use the 'sandwich technique' (to be differentiated from the glue sandwich technique mentioned earlier) [21]. This involves coil deployment distally (at the level of the pubic ramus), followed by a sclerosant and then sealing that with another coil. This prevents reflux of the sclerosant, and at the same time, avoids nontarget sclerotherapy. However, as mentioned previously, this proximal coil may preclude reentry in the event of recurrence.

This technique is reported to have a high success rate (as high as 91%) and a recurrence rate (of approximately 20%) [12, 22].

Our Technique—The Catheter–Wire Combination; the Key to Success

When accessing the left ISV, right femoral access is preferred. However, for right ISV embolization, a jugular access may be easier. The use of ultrasound vastly increases the ease and safety of venous access.

Under ultrasound guidance, the selected vein is punctured using a 22G Angiocath or micropunc-

ture needle and a 5 or 6 French vascular sheath is placed. A 4 Fr C2 catheter is used to select and catheterize the left renal vein and then the orifice of the left ISV. A 4F Bernstein catheter may be beneficial in children. If the caliber of the ISV does not accommodate the 4F catheters, a microcatheter may be used (Terumo Progreat being our preference). 0.035-inch hydrophilic wires in regular or stiff form are the wires of choice. The Progreat microcatheter comes prepackaged with its own wire. A guiding catheter may be of benefit to enhance vascular catheter stability.

After reaching the distal ISV, a pull-back venogram is advised to assess for collaterals. Embolization MUST start below the level of the first visible collateral to minimize chances of recurrence. This usually starts with the distal coil to prevent sclerosant propagation distally, followed by 5–10 cc of 3% STS mixed with contrast. After forming the contrast-sclerosant column and allowing the sclerosant to sit for approximately 10 minutes, topping up if necessary, the proximal coil is deployed. This is the final layer of the sandwich. Our personal preference is not to deploy the proximal coil. This facilitates reentry if a second embolization session is needed.

The vascular sheath is then removed and a vascular occlusion device used, or the site is compressed for 10 minutes. No dressing application is necessary (Figs. 19.2 and 19.3).

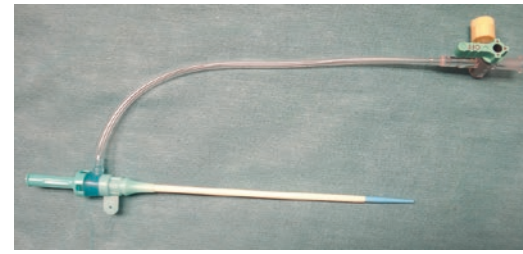


Fig. 19.2 8 French vascular introducer sheath. The Terumo Radifocus vascular introducer sheath (Terumo, Japan). Our preference is to use this vascular access sheath due to its hydrophilic properties

Fig. 19.3 6F Cobra catheter. Renal access Cobra catheter (Cook Medical, USA). This catheter is an excellent choice for adult patients as its shape and stiffness work well with gonadal vein selection. The smallest size available is 6 French, which may be too large for pediatric patients

Postprocedural and Follow-Up Care

Immediately postprocedure, bed rest without bathroom privileges is recommended for 4 hours. Bathroom privileges can be allowed in the following 2 hours, with careful monitoring of the puncture site with mobilization. The patient may be discharged 6 hours after the procedure with restriction of their activity to only light ambulation and no exercise for 2 weeks. Analgesics and anti-inflammatories can be prescribed on a PRN basis.

The patient is seen in clinic 2 weeks later and ultrasounds are planned for 3 and 6 months later. Reduction of the varicocele to a grade 1 (Sartechi el al [3].) as per the sonographic classification mentioned above would indicate a successful treatment and an excellent outcome. A grade 2 varicocele would prompt a third follow-up at 12 months to rule out recurrence or failure. No improvement at all at 3 months would denote clinical failure of the procedure. Semen parameters may be assessed at the same intervals and assessed for improvement.

Complications

Complications to varicocele embolization are quite rare and include general angiography-related issues including groin hematoma formation, contrast agent-related reactions, fever, and nausea. This usually occurs in less than 1% of patients. Venous thrombosis may occur in less than 5% [9, 23].

Procedure-specific complications include thrombosis of the pampiniform plexus in 1–5% of patients. The symptoms are similar to acute epididymo-orchi-

tis and include pain and swelling and require analgesia and antibiotics. Hydrocele is believed to occur less than when surgery is performed [24].

Outcomes

Technical success rates range from 90% to 97% regardless of the embolic agent. The recurrence rate ranges between 4% and 27% [9, 23]. Failure is usually technical and has a range of 1–12% [17, 25, 26]. Complications are usually lower than surgical intervention [24, 27]. Some authors also consider embolization as a more cost-effective method due to the shorter hospital stay [28]. Several studies have found no difference in pregnancy outcome between surgery and embolization [17, 28] Cassidy et al. have performed one of the largest studies comparing embolization to surgery and recommend surgery for bilateral varicoceles and embolization for unilateral left-sided varicoceles [29]. This is attributed to high failure rate in embolizing right-sided varicoceles in their cohort. This may well be operator, or technique-related; however, given the 'relatively' large sample size, this cannot be disregarded.

Surgery

With different institutions having different academic interests and financial priorities, varicocele management is highly variable. Our preference is that the first line of varicocele management be surgical and embolization be reserved for patients with recurrence or patients refusing surgery. This is due to the high success rate within our urology team and the availability of interventional radiologists. This may differ from one institution to another.

Surgery is the most common form of varicocele management and can be performed as a day surgery, overnight stay, or a few days of inpatient stay. It is performed by retroperitoneal or trans-inguinal ligation of the ISV. Recurrence is usually due to venous collateralization and leads to persistence of the varicocele. In these cases, meticulous venography with relatively higher pressure hand injections is warranted to try and demonstrate the presence of any collaterals as they may not be visible with regular or low pressure injections.

The surgical recurrence ranges between 0% and 28% [17]. It is lower for laparoscopic approaches (7–9%) [30], and even lower when microsurgery is performed (0–3%) [31, 32] (Figs. 19.4, 19.5, 19.6, and 19.7).

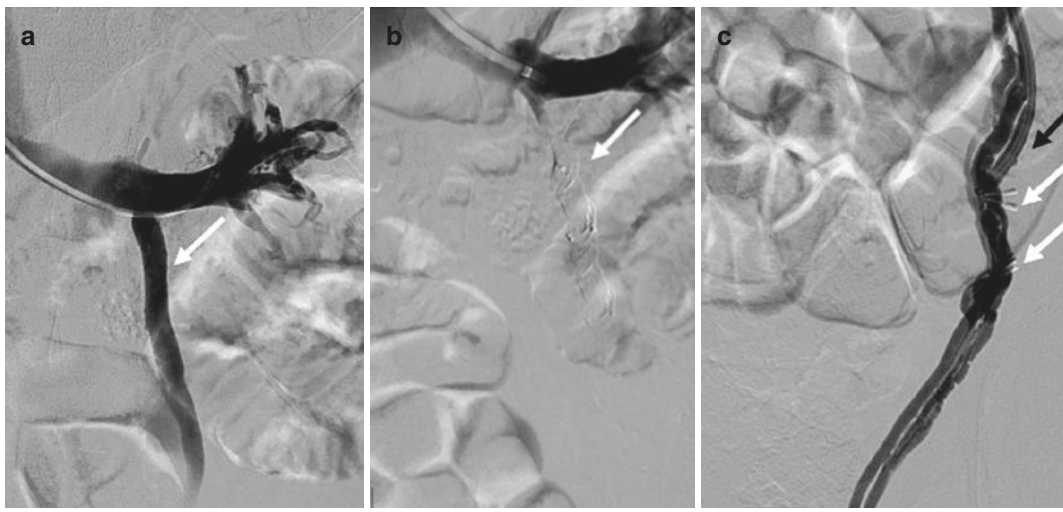


Fig. 19.4 Embolization of a recurrent Bähren type III varicocele in a 23-year-old patient. Pre-embolization (**a**) and post-embolization (**b**) images of a recurrent Bähren III varicocele. In Fig. 19.4a, there is obvious reflux into the left ISV on venography (white arrow). There is duplication of the ISV caudally (**c**, black arrow) with coalescence into a single trunk at the renal vein (**a**). Post-embolization with vascular plugs (**b**). The plugs (white arrow) have completely occluded flow from the ISV draining into the left renal vein

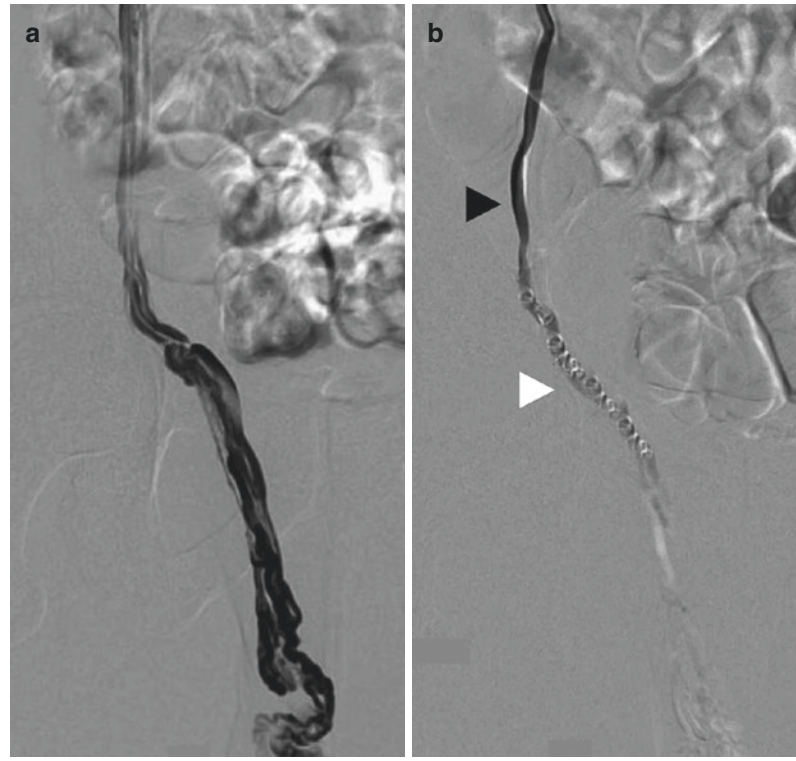


Fig. 19.5 Right-sided varicocele in a 15-year-old patient pre- (**a**) and (**b**) post-embolization with coils deployed distally (white arrow head) and a column of STS-contrast mixture above (black arrow head)

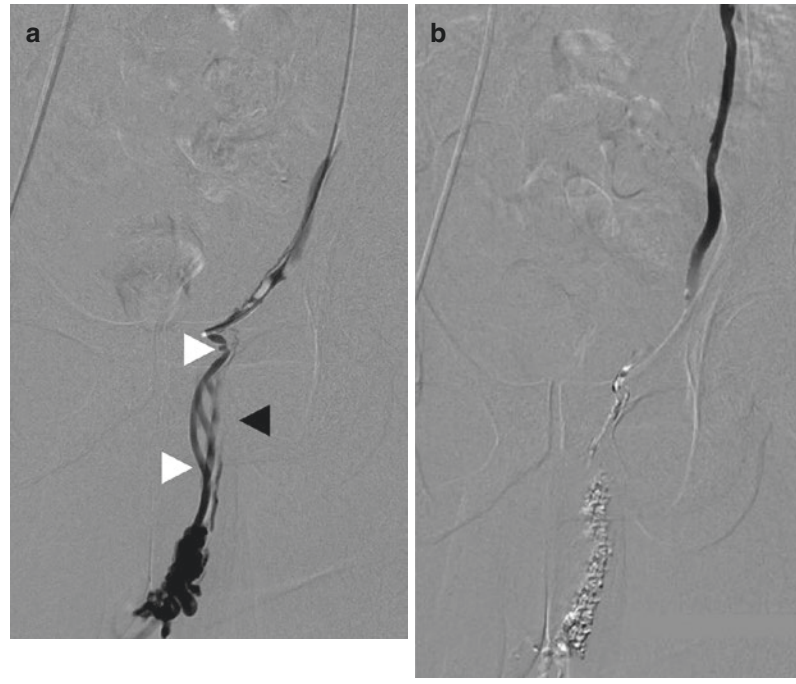


Fig. 19.6 (**a**, **b**) Left-sided varicocele in a 37-year-old patient that has recurred after glue embolization. A second glue embolization session was required. There was no evidence of recurrence 6 months later (**b**). The previously embolized vein (black arrow head) and new ISV collateral vein formation (white arrow head) are seen in the pre-embolization images (**a**)

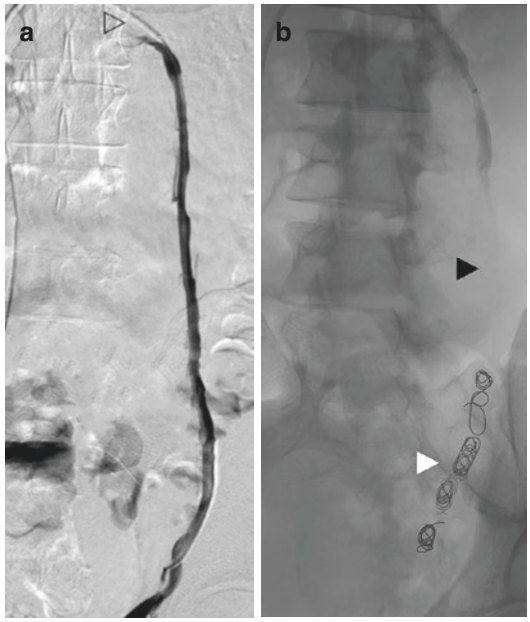


Fig. 19.7 Left-sided varicocele in a 17-year-old patient pre (**a**) and (**b**) post-embolization with coils deployed distally (white arrow head) and a column of STS-contrast mixture above (black arrow head). A 6F guiding catheter was used to assist with catheter stability (clear arrow head)

Review Criteria

An extensive search of studies examining varicocele anatomy and image-guided sclerotherapy/embolization of varicoceles was performed using search engines such as OVID, Google Scholar, and PubMed. The overall strategy for study identification and data extraction was based on the following key words: "varicocele", "sclerotherapy", "embolization", "interventional", "image guided", and "anatomy", as well as the names of specific sclerosants. Articles published in languages other than English were also considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included.

Multiple Choice Questions and Answers

1. The Best Practice Policy Committee of the American Urological Association and the Practice Committee of the American Society for Reproductive Medicine recommend that adolescents and young men with the following should undergo annual follow-up monitoring, except.
   (a) A varicocele with a normal-size ipsilateral testicle
   (b) A varicocele with normal semen analysis
   (c) A varicocele with a normal-size ipsilateral testicle AND a normal semen analysis
   (d) **Bilateral varicoceles**.
2. The following embolic agents have been used for varicocele embolization, except.
   (a) STS
   (b) Coils
   (c) Glue
   (d) **Contrast**
   (e) Vascular plugs
3. Bed rest after varicocele embolization is required for.
   (a) 2 hours without bathroom privileges followed by another 2 hours with bathroom privileges
   (b) **4 hours without bathroom privileges followed by another 2 hours with bathroom privileges**
   (c) 2 hours without bathroom privileges followed by another 4 hours with bathroom privileges
   (d) 4 hours without bathroom privileges followed by another 4 hours with bathroom privileges
4. The normal variants of the internal spermatic vein include the following, except.
   (a) The right ISV can drain into multiple terminating veins in the IVC and the renal vein in 16%.
   (b) The left ISV can drain into multiple terminating veins in the left renal vein in 20% of cases.

(c) **There is no communication between the retroperitoneal, peritoneal, adrenal, and portal vein collaterals**.

(d) The left and right ISVs at the level of L3 may communicate. A circum-aortic left renal vein may be seen in 9% of patients.

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Robotic-Assisted Microsurgical Varicocelectomy

20

Mohamed H. Etafy, Richard A. Mendelson, and Sijo J. Parekattil

Key Points

- The use of a robotic microsurgical treatment modality enabled surgeons to perform procedures on otherwise inaccessible areas of the male anatomy.
- The field of Assisted Robotic Microsurgery (ARM) has allowed for an enormous leap forward in terms of male fertility treatment during the past 3 decades.
- When used to perform varicocelectomy, the use of robotic microsurgical modality generates a shorter duration of operative time once the learning curve is accounted for and decreases postoperative complications significantly.
- Use of the Robot Microsurgical Modality eliminates or mitigates human factors such as tremor and fatigue and allows for additional instruments, magnification, and improved dexterity.
- Use of the DaVinci Microsurgical modality allows for the use of micro-Doppler probes that decrease the risk of damage to testicular arteries during varicocelectomy or male fertility treatment operations.

Robotics in Male Infertility

The year 1970 marked the first use of a microscope in the performance of surgical procedures. The use of the microscope gave birth to the "New Revolution", or a zeitgeist in surgical procedures [1], as the microscope allowed surgeons to operate on smaller structures that were previously considered inoperable or were accompanied by far higher risk associated with surgery.

The introduction of the DaVinci robotic platform for laparoscopic surgery has been associated with shorter hospital stay and decreased peri-operative morbidity. This was a huge ergonomic advancement for laparoscopic surgeons. Early groups studied the feasibility and efficacy of potentially using this robotics platform for microsurgery. Schiff et al. [2] found that robotic-assisted vasovasostomy and vasoepididymostomy were significantly faster than pure

M. H. Etafy (✉)
Al-Azhar University Hospital, Assiut, Egypt

Department of Urology, PUR Clinic, South Lake Hospital, Clermont, FL, USA

R. A. Mendelson
Department of Graduate Psychology, Keiser University, Ft. Lauderdale, FL, USA

S. J. Parekattil
Personalized Urology & Robotics, The PUR Clinic & University of Central Florida, Clermont, FL, USA

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microsurgical techniques in an animal model with comparable outcomes.

The DaVinci robotic platform offers ergonomic and surgical efficiency advantages to the microsurgeon, in that it provides a three-dimensional high-definition magnified view (15×) with the use of three surgical arms. There is a learning curve to get used to operating with three arms (versus two in standard microscopy), but this allows the microsurgeon to operate without the need of a skilled microsurgical assistant. The microsurgeon has complete control of the field [3].

The fourth arm also allows the microsurgeon to utilize imaging and sensing tools such as micro-Doppler probes or micro Ultrasound probes in real-time, while the surgeon is operating with the other two arms. This is simply not achievable in microsurgery without the use of a skilled surgical assistant. This additional imaging or sensing modalities can be integrated into the surgical console so that the microsurgeon can also see or hear these inputs in real-time [4].

Ten to fifteen percent of couples who are attempting to conceive encounter fertility issues. Fifty percent of these couples may have some type of male factor contribution to the cause of infertility. The presence of a varicocele leads to a two-fold increase in the likelihood of having abnormal semen analysis parameters in men seeking infertility treatment [3]. This may be a result of the increased temperature of scrotal contents due to increased blood volume in the tissues of that area. Varicocelectomy can lead to significant improvements in semen analysis parameters. A meta-analysis indicates significant improvements in sperm count and motility regardless of the varicocelectomy technique [4].

Results from a prospective, randomized controlled trial (RCT) from Saudi Arabia compared subinguinal microsurgical varicocele repair to observation [5]. A total of 145 participants had follow-up within 1 year; natural pregnancy was achieved in 13.9% of controls compared with 32.9% of treated men (odds ratio 3.04). This study provides evidence of the superiority of varicocelectomy over observation in infertile men with palpable varicoceles and impaired semen quality. In fact, work by multiple research groups indicates that treatment of varicocele through microsurgery improved semen parameters in all patients [4, 6].

Shu et al. [7] published the initial study showing the safety, feasibility, and comparable outcomes of robotic assistance in subinguinal microsurgical varicocelectomy in 2008. They described elimination of tremor, and the stable, ergonomic platform as benefits of the robotic approach. Parekattil et al. [5] further explored this technique in a canine spermatic cord as they performed a prospective RCT of microscopic varicocelectomy vs robot-assisted microsurgical varicocelectomy (RAVx) in a canine varicocele model. In all, 12 canine varicocelectomies were randomized into two arms of six: standard microscopic varicocelectomy vs RAVx. There were no vessel injuries or knot failures in either group. There was no significant difference in setup duration between the robot and operative microscope. They found a significantly faster operative time with RAVx when compared to the typical microsurgical approach (RAVx mean duration 9.5 min, while in standard microscopic varicocelectomy the mean duration was 12 min).

Mechlin and McCullough [8] reported results of their initial experience with RAVx. They retrospectively reviewed surgical outcomes for patients who underwent varicocelectomy either by a standard microsurgical approach (34 patients) or RAVx (33 patients) by a single surgeon at an academic center. They reported no significant difference in operative time when comparing RAVx to standard microsurgical varicocelectomy (57 min for RAVx vs 49 min for the microscopic group). They concluded also that the learning curve and operative time were progressively diminishing in their most recent cases. McCullough et al. [9] reported a similar conclusion when they compared both groups and found that their operative time decreased in their recent cases.

It is likely that the overall clinical outcomes of the robotic versus the pure microsurgical technique are comparable. The difference is in surgical efficiency. The robotic platform is simply a surgical tool, and as with any tool, its main purpose is to allow the microsurgeon to operate more ergonomically and efficiently. This can be seen in any industry that incorporates robotics, such as the automotive industry. Robotics simply allows us to achieve similar outcomes with much higher throughput.

Technique for Robot-Assisted Microsurgical Varicocelectomy

A 2–3 cm subinguinal incision is made over the location of the external inguinal ring. The spermatic cord is carefully dissected and then raised through the skin incision. A 1/2" inch Penrose drain is placed under the cord to keep it elevated. A sterile tongue blade is placed through the Penrose drain under the cord to further elevate and spread the cord. The robot is positioned from the patient's right side (Fig. 20.1). The black diamond micro forceps are used in the right robotic arm, the micro bipolar forceps in the left arm, and the curved monopolar scissors in the fourth arm. The cremasteric sheath of the spermatic cord is then incised to separate the cord structures (Fig. 20.2). Real-time intraoperative Doppler ultrasound is utilized to localize the testicular artery and ensure that no injury occurs to this vessel (Fig. 20.3). Enlarged veins are carefully dissected and then ligated using 3-0 silk suture ties. Doppler ultrasound verification of each vessel before it is ligated is performed to ensure that no arteries are ligated (Fig. 20.4). The curved monopolar scissors or Potts scissors in the fourth arm are used to cut the vessels after being tied. The tongue blade is removed from within the Penrose. The Penrose is now carefully removed, and the spermatic cord is released. The testicle is gently pulled down to retract the spermatic cord completely into the incision. The skin incision is closed at the subcutaneous layer using a 3-0 polyglactin suture. The skin is closed using a running subcuticular 4-0 monocryl suture and skin glue.

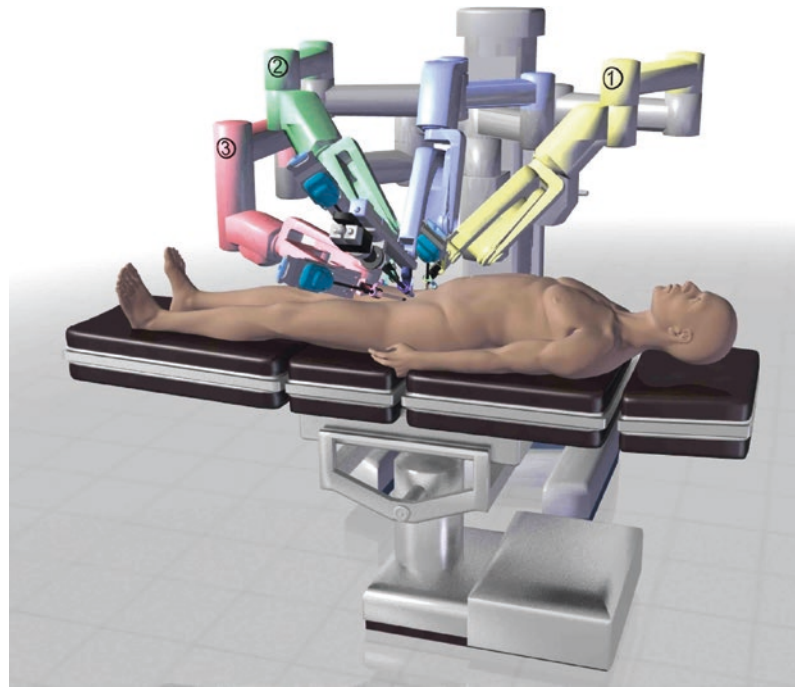

Fig. 20.1 Operative setup for robot-assisted microsurgical procedures

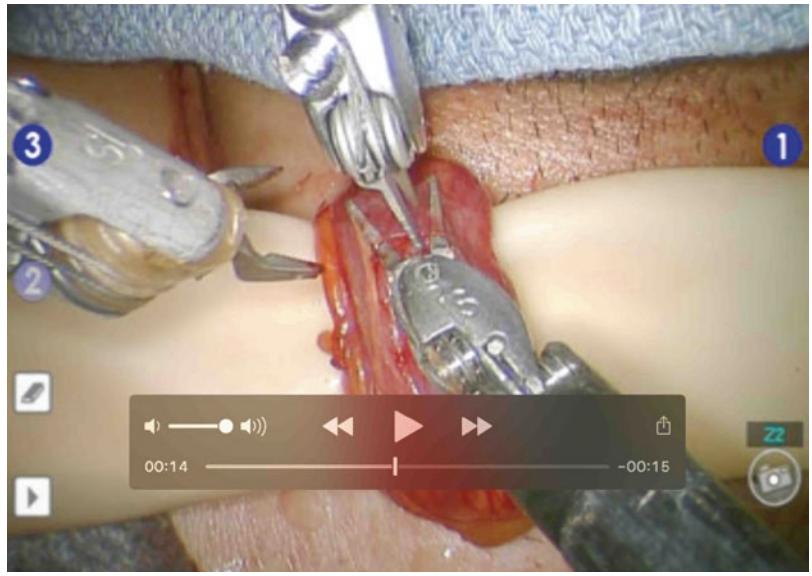

Fig. 20.2 Incision of cremasteric sheath

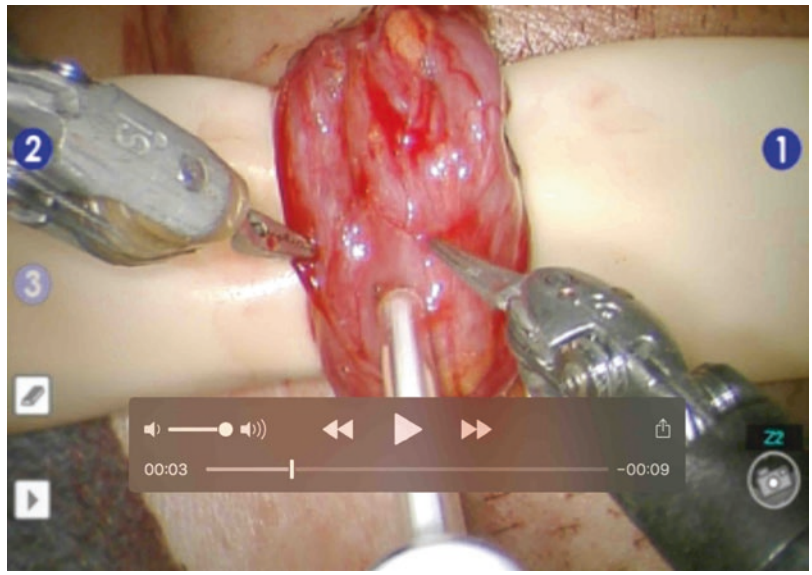

Fig. 20.3 Usage of intraoperative Doppler ultrasound

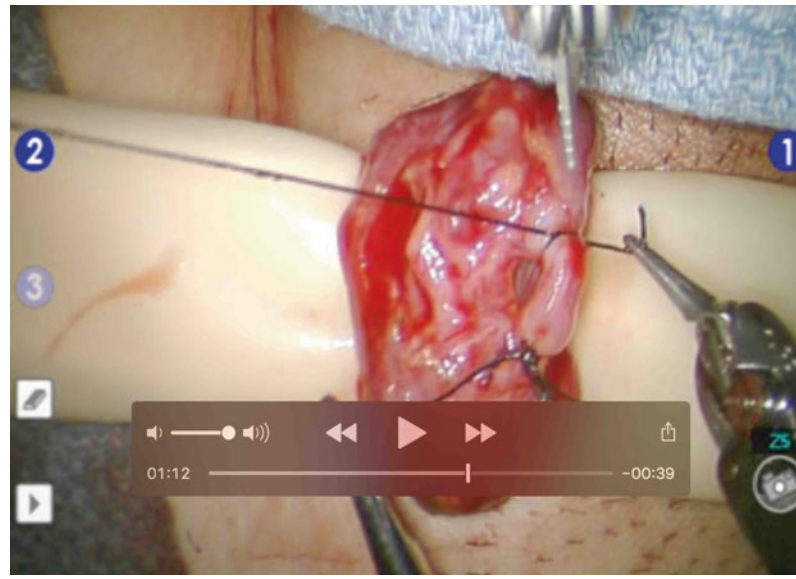

Fig. 20.4 Ligation of dilated veins

Postoperative Management

Robotic varicocelectomy is generally performed as an outpatient procedure. A scrotal support is placed prior to awaking the patient. The patient is asked to use this support for 2–3 weeks after surgery. The patient is instructed to have limited activity and have bed rest for about 1 week after surgery. No strenuous activity or heavy lifting is allowed for 4 weeks postoperatively. All patients are provided prescriptions for narcotics for a brief period and antibiotics (Keflex) for a few days. Patients are instructed to utilize ice packs (30 min on and off) for the first week post-op to minimize the use of narcotics.

Reduction of Complications

Coccuzzo et al. [10] have recently shown that the systematic use of intraoperative Doppler during microsurgical varicocelectomy can significantly decrease the risk of inadvertent testicular artery injury. Thus, we routinely utilize this modality during varicocelectomy to optimize patient safety.

Currently, there are two available micro-Doppler US probes: VTI (Vascular Technology Inc., Nashua, NH, USA) provides an easy-to-use, audible and disposable micro-Doppler probe (Fig. 20.5) and Aloka (Hitachi-Aloka, Tokyo, Japan) has a micro-Doppler US probe (Fig. 20.5) that provides full-depth US imaging of the spermatic cord with Doppler flow sensing as well. The output from this probe can be sent directly to the surgeon console to provide real-time simultaneous imaging while the surgeon is operating.

Outcomes

From June 2008 to September 2014, 264 robotic-assisted varicocelectomy cases were performed in 220 patients. Indications for the procedure were the presence of a grade 2 or 3 varicocele and the following conditions: azoospermia in 20 patients, oligozoospermia in 63 patients, and chronic orchialgia with or without oligozoospermia in 137 patients. The median duration per side was 25 min (10–80). Median follow-up was 36 months (1–76). Eighty percent with oligozoospermia had a significant improvement in sperm count or motility, 30% with azoospermia converted to oligozoospermia, and 91% of the testicular pain patients had a significant reduction in pain (82% of these patients had targeted denervation of the spermatic cord in addition to varicocelectomy). Two recurrences or persistence of varicocele occurred, one patient developed a small postoperative hydrocele, two patients had

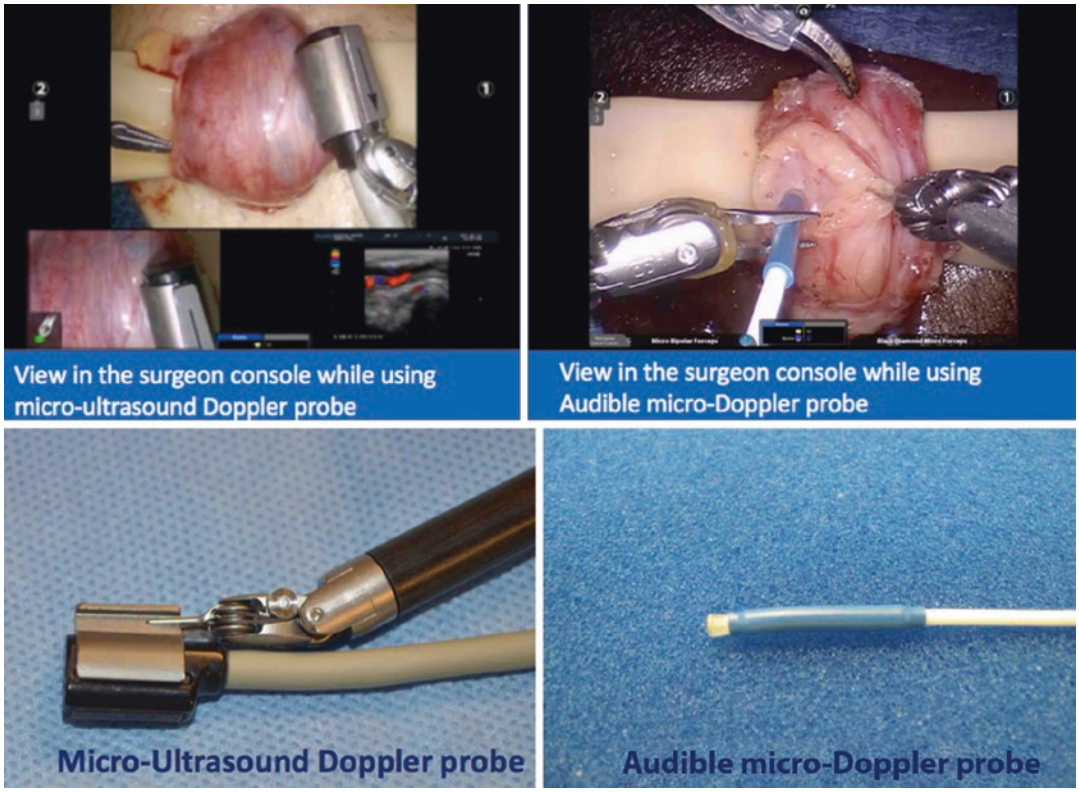

Fig. 20.5 Intraoperative Doppler US systems (left side audible micro-Doppler, right side visual micro-Doppler)

postoperative scrotal hematomas, and five patients had wound seroma (treated conservatively). The fourth robotic arm allowed the surgeon to control one additional instrument during the cases, decreasing reliance on the microsurgical assistant. The fourth arm also enabled the surgeon to perform real-time intraoperative Doppler mapping of the testicular arteries while dissecting the veins with the other arms if needed.

Cost

True cost of a robotic varicocelectomy is a complex calculation. In high-volume robotic multi-specialty surgical hospitals, the cost to perform a robotic microsurgical case may be comparable to investing in a pure microsurgical platform and utilizing a microsurgical assistant (mostly obviated in the case of robotic cases due to the extra robotic arm that the surgeon controls) as shown in Fig. 20.6. In a hospital that does not have a robot, but has a surgical microscope, the upwards of 2.5 million dollar capital investment to get a robot may not be justified for just one microsurgeon. However, the majority of hospitals in the US now have robotic platforms and they are utilized by surgeons from varying specialties (General Surgery, GYN, ENT, Urology and Cardiac Surgery). In fact, robotic time is difficult to procure. The three surgeons in this pro-debate for robotic microsurgery are at high-volume robotic surgical centers. The mean robot utilization at these centers is 105%, and the mean utilization of the microscope is 31%. The cost per case for robotic cases is comparable to microscopic cases due to the high utilization of the robot by multiple surgeons across specialties and the low utilization of the microscope (Fig. 20.6). Figure 20.6 illustrates cost analysis models for two scenarios and demonstrates how the cost per case could be comparable between robotic and microscopic cases based on case volumes. There is an additional mean cost of $450 for disposables for robotic cases over microscopic cases.

Cost analysis model assuming 96 microscopic cases a year		
	Robot	**Microscope**
Purchase price	$2,500,000	$200,000
Service contract	$250,000	$20,000
Days in use a year (48 weeks)	240	48
Cases per day	3	2
Cases per year (48 weeks)	720	96
Total cases over 5 years	3600	480
Cost per case over 5 years	**$763.89**	**$458.33**
Cost analysis model assuming 24 microscopic cases a year		
	Robot	**Microscope**
Purchase price	2500000	200000
Service contract	250000	20000
Days in use a year (48 weeks)	240	24
Cases per day	3	2
Cases per year (48 weeks)	720	48
Total cases over 5 years	3600	240
Cost per case over 5 years	**$763.89**	**$916.67**

Fig. 20.6 Capital investment to cost per case analysis for robotic versus microscopic cases over 5 years: scenarios for comparable cost

Facility	**Self pay pricing ($)**				
		Unilateral varicocelectomy			
		Facility charge	Surgeon fee	Anesthesia	Total
Robotic Microsurgical Centers:					
Institution A	With anesthesia in OR (VV or VE)	7400	1500	1000	9900
Institution B	With anesthesia in OR (VV or VE)	3158	1730	900	5788
Institution C	With anesthesia in OR (VV or VE)				18000
Mean robotic self pay cost to patient					**11229**
Pure Microsurgical Centers:					
Institution A	With anesthesia in OR (simple)	23000	8000	5000	36000
	With anesthesia in OR (complex)	27000	12000	6000	45000
	Under Local				
Institution B	With anesthesia in OR (simple)	4875	1486.40		6361.40
	With anesthesia in OR (complex)	5927	1486.40		7413.40
Institution C		2000	325	500	2825
Mean micro self pay cost to patient					**19520**

Fig. 20.7 Self-pay pricing comparison for robotic versus microscopic vasectomy reversal and varicocelectomy

Varicocelectomy is a unique procedure in that patients may pay for these procedures out of pocket. Self-pay pricing for surgical procedures is one measure of how much a procedure actually costs. Figure 20.7 illustrates self-pay pricing for varicocelectomy procedures at six institutions

based on a survey of pricing in US dollars on 7/25/18 in the United States and Canada.

The data illustrate that robotic microsurgery may not be more expensive than pure microsurgery. The cost to the patient is based more on profit margins, facility costs, and less so on the technology that is being utilized. Use of the robotic platform standardizes the OR staff processes, since they are simply using the same tool and setup for a number of different types of cases. Since a microsurgical assistant is not needed, less staffing is involved for robotic cases.

Conclusion

Robotic-assisted microsurgical varicocelectomy is an effective, feasible, and safe treatment option. It offers comparable clinical outcomes in studies from three different centers. It offers a more efficient and ergonomic platform for the microsurgeon.

Review Criteria

The search strategy was conducted in accordance with Cochrane guidelines and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A search strategy was conducted in MEDLINE, PubMed, and the Cochrane electronic databases (from 2000 to present) to identify studies that included both robotic and varicocelectomy. The search was conducted using the following keywords: "robotic," "robot-assisted," and "varicocele." Medical Subject Heading (MeSH) phrases included ("Robotic" [MeSH]) AND ("Varicocele" [MeSH]), ("Robotics" [Mesh]) AND ("Andrology" [MeSH]), ("Varicocelectomy" [MeSH]) AND ("Robotics" [MeSH]). Both retrospective and comparative studies were included. The language of the articles was restricted to English and articles were excluded if the study did not include both male infertility and robotics.

Multiple Choice Questions and Answers

1. When was operative microscopy started to be used in varicocelectomy operations?
   (a) Early 50s
   (b) Early 60s
   (c) **Early 70s**
   (d) Early 80s
2. What robotic platform is used for microsurgical procedures instead of operative microscope?
   (a) **DaVinci robotic platform**
   (b) Aesop robotic platform
   (c) Sport robotic platform
   (d) Raven robotic platform
3. During the process of a varicocelectomy procedure, the surgeon must be aware that prior research indicates that most patients have multiple testicular arteries located where?
   (a) Subinguinal area
   (b) **Spermatic cord**
   (c) Cremaster muscle
   (d) Vas deferens
4. In order to avoid damage to testicular arteries, which tool could be used?
   (a) Micro-TESE extractor
   (b) Robotic scissors
   (c) Microsurgical forceps
   (d) **Micro-Doppler US probe**
5. What is the magnification capability of the da Vinci surgical platform for microsurgical procedures?
   (a) 3x
   (b) 6x
   (c) 9x
   (d) **15x**

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Effect of Varicocele Treatment on Conventional Semen Analysis

21

S. V. Krishna Reddy and Ahammad Basha Shaik

Key Points

- Varicoceles can present in up to 40% of men presenting with infertility, and published literature support the findings that varicocele adversely affects spermatogenesis.
- The incidence of varicocele was 25.4% in men with abnormal semen parameters compared with 11.7% in men with normal semen.
- Varicocele repair for infertility is mainly indicated in patients with clinically palpable varicocele and abnormal semen parameters.
- In a large number of studies, sperm concentration, morphology, and motility improved in 42%, 57%, and 29% of patients, respectively, after varicocele repair.
- Cut-off values of >8 million/mL and >18% for sperm density and progressive motility, respectively, in men with clinical varicoceles indicate a successful outcome after varicocele repair.

Introduction

Varicocele is the most frequently identifiable and treatable cause of male infertility. The percentage of clinically evidenced varicocele in young adult subjects varies from 9% to 23%, as reported by the most recent case studies. Furthermore, varicocele can be observed in over 40% of infertile males [1]. In a large series of 9034 infertile men, the World Health Organization (WHO) reported that the incidence of varicocele was 25.4% in men with abnormal semen parameters compared with 11.7% in men with normal semen [2]. Varicoceles are currently the most common abnormality identified in men being evaluated for infertility. The surgical repair of the varicocele as a treatment for infertility was first reported in 1952, when the Edinburgh surgeon Selby Tulloch demonstrated the restoration of fertility following excision of bilateral varicocele in an azoospermic patient [3]. Since then, thousands of studies on the diagnosis and surgical correction of varicoceles

S. V. K. Reddy (✉)
Department of Urology, Narayana Medical College & Hospital, Nellore, Andhra Pradesh, India
e-mail: krishnareddysv@narayanamedicalgroup.com

A. B. Shaik
Department of Community Medicine, Narayana Medical College & Hospital, Nellore, Andhra Pradesh, India

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have appeared in the literature. Unfortunately, this entire body of experimental evidence has not been able to either identify the mechanism of spermatogenesis impairment or explain why surgical correction improves semen parameters. Possible reasons for the improvement in semen parameters are decreases in oxidative stress, increases in semen antioxidants, and improvements in sperm quality. Almost all varicocele are detected after puberty. The higher incidence in secondary infertility implicates varicocele in producing a progressive decline in testicular function over time involving both spermatogenesis and steroidogenesis. Several theories have been formulated to explain the testicular impairment caused by varicocele, however, none of them can completely explain the variable modulating effect of varicocele on male fertility. Recently, the oxidative stress theory has emerged as an important contributory factor due to findings of an association between elevated reactive oxygen species and impaired sperm function in men with varicocele [4]. In clinical practice, most reports show persistent abnormality of sperm count, motility, or morphology [5]. The researchers still debate as to what extend varicocele affects semen parameters. Initially, sperm concentration is not seriously affected; though later all three sperm parameters can gradually deteriorate, resulting in azoospermia in very few cases. Although many individual studies report improvement after varicocele repair, there are still conflicting opinions as to whether a varicocele repair improves fertility. Physical examination is the reference standard to diagnose varicoceles in subfertile men. A clinical varicocele is defined as a palpable elongated, dilated, and tortuous testicular pampiniform plexus of veins in the spermatic cord [6]. Additional radiologic imaging is not essential for detection of subclinical varicocele, because only a varicocele detected by physical examination should be considered potentially significant [7, 8]. While all guidelines recommend physical examination as the cornerstone of varicocele diagnosis, the EAU guidelines state that it should be complemented with color duplex ultrasonography. When clinical palpable varicocele coexists with impaired semen quality, surgical repair may potentially restore spermatogenesis and fertility.

Threshold Values for Semen Analysis [*WHO Lower Reference Limits*]

Minimum requirements for semen parameter standards were followed by publications from the World Health Organization (WHO) in 1980, 1987, 1992, and 1999 [9–11]. However, the so-called normal values provided by the WHO manuals for the basic semen parameters, viz., volume, qualitative and quantitative motility, and morphology, were mostly obtained through studies performed on so-called fertile populations (WHO, 1987 WHO, 1992). In its latest fifth edition (WHO 2010) [9] the semen analysis reference values are markedly lower than those of previous editions. The lower reference limits in the manual aimed to provide the clinicians with evidence-based thresholds while estimating the relative fertility of a given patient. The adoption of the new WHO reference values will likely lead to more men being classified as "fertile," which is of particular importance for gynecologists who rely on semen analysis alone as a surrogate measure for male fertility. Application of the new WHO reference values might lead to patients earlier deemed to be candidates for varicocele repair now be considered ineligible for treatment if their semen parameters are above the fifth edition values. Many authors thereafter in a series of reports have questioned the validity of the newly released reference values [10–14] (Table 21.1) [15]. These values will have to be taken while analyzing the reports on semen parameters and varicocele. As there is a considerable overlap between the semen characteristics of fertile and subfertile men, there is no parameter that can be used to provide prognostic information about the fertility potential. However, since many articles are dating back to periods even before 2000, other threshold values will also be taken while analyzing the reports hence further research is required to fully understand the impact of this change on the association

Table 21.1 Cut-off reference values for semen characteristics as published in consecutive WHO manuals

Semen Characteristics	WHO (1980)	WHO (1987)	WHO (1992)	WHO (1999)	WHO (2010)
Volume (mL)	ND	>2	>2	>2	1.5
Sperm count (10^6/mL)	20–200	>20	>20	>20	15
Total sperm count (10^6)	ND	>40	>0.40	>40	39
Total motility (% motile)	>60	>50	>50	>50	40
Progressive motility[a]	>2[b]	>25%	>25% (grade a)	>25% (grade a)	32% (a + b)
Vitality (% alive)	ND	>50	>75	>75	58
Morphology (% normal forms)	80.5	>50	>30[c]	(14)[d]	4[e]
Leukocyte count (10^6/mL)	<4.7	<1.0	<1.0	<1.0	<1.0

Based on data from Ref. [9–11]
Lower reference limits generated from the lower fifty centile value
ND not defined
[a]Grade a = rapid progressive motility (>25 um/s); Grade b = slow/sluggish progressive motility (5–25 um/s); Normal = 50% motility (grades a + b) or 25% progressive motility (grade a) within 60 min of ejaculation
[b]Forward progression (scale 0–3)
[c]Arbitrary value
[d]Value not defined but strict criterion is suggested
[e]Strict (Tygerberg) criterion

between varicocele and semen parameters. The percentage changes in the parameters will also be taken into account in interpreting the effect of varicocele repair. The most recent Practice Committee report on varicocele by the American Society for Reproductive Medicine (ASRM) acknowledged the limitations of routine semen analysis and included the presence of an abnormal sperm function test as an indication for treatment [16]. The observed pooled effect size does not seem to be affected by the WHO laboratory manual edition used for the examination of human semen. With the introduction of intracytoplasmic sperm injection (ICSI), the role of the standard semen analysis is becoming a greater point of discussion. Because these lower thresholds have a much higher positive predictive value, they suggest that thresholds of <5% normal sperm morphology, a concentration <15 × 10(6)/ml, and a motility <30% should be used to identify the subfertile male. The lower threshold for morphology also fits in vitro fertilization and intrauterine insemination data.

The Impact of Varicocelectomy on Semen Parameters

Since the classic work of Macleod (1969) [17, 18], who described an association among infertility, abnormal semen parameters, and varicocele, a multitude of studies have examined varicocele by various angles. Nonetheless, the exact mechanisms implicated in the pathophysiology of varicocele on male fertility are not fully understood. Conventional semen parameters analysis has been an important laboratory examination during the initial evaluation of male factor infertility. Semen with lower values other than the values mentioned before was considered as abnormal. An early study conducted by the World Health Organization (WHO) involving 9034 men demonstrated that both sperm concentration and motility were lower in men with varicocele than in men without varicocele [12]. In a recent large-scale study of 7035 healthy young men from general European populations, the presence of varicocele was associated with poorer semen quality, even in men with grade-I varicocele (Table 21.2 and Fig. 21.1) [19]. In 2010, the WHO announced the first semen criteria based on a large study of fertile men across seven countries [12, 20]. In that updated fifth edition of the WHO manual, novel methods for measuring ejaculate volume by weight and assessing sperm morphology by strict criteria were incorporated [12]. In addition, changes in the methods for assessing sperm count, sperm motility, and quality control routines were included (Table 21.3) [21]. Studies suggest that varicocele repair significantly increases sperm parameters, including sperm con-

Table 21.2 Unadjusted seminal parameters of 7035 young men from the general European population

	Median (5–95)	Overall between varicocele groups	Specific group to on varicocele
Semen volume, ml			
All men	3.1 (1.2–6.1)	0.006	
No varicocele	3.1 (1.2–6.0)		
Grade 1 varicocele	3.4 (1.3–6.4)		0.001
Grade 2 varicocele	3.4 (1.3–6.5)		0.2
Grade 3 varicocele	3.1 (1.3–6.0)		0.2
Sperm concentration,million/ml			
All men	50 (4–173)	<0.001	
No varicocele	51 (5–177)		
Grade 1 varicocele	48 (4–163)		0.002
Grade 2 varicocele	40 (2–145)		<0.001
Grade 3 varicocele	28 (1–142)		<0.001
Total sperm count, million			
All men	157 (10–576)	<0.001	
No varicocele	159 (12–581)		0.2
Grade 1 varicocele	168 (8–544)		<0.001
Grade 2 varicocele	136 (5–586)		<0.001
Grade 3 varicocele	85 (1–456)		
Normal morphology,%[a]			
All men	8.0 (1.0–18.0)	<0.001	
No varicocele	8.0 (1.0–180.0		0.1
Grade 1 varicocele	8.0 (1.0–17.7)		0.01
Grade 2 varicocele	7.5 (0.5–18.6)		<0.001
Grade 3 varicocele	5.0 (0.0–15.5)		
Total normal spermatozoa, million[+]			
All men	11 (0–70)	<0.001	0.046
No varicocele	11 (0–71)		<0.001
Grade 1 varicocele	13 (0–68)		<0.001
Grade 2 varicocele	8 (0–63)		
Grade 3 varicocele	5 (0–50)		
Progressively motile (A + B),%		0.001	0.5
All men	58 (25–77)		<0.001
No varicocele	59 (26–77)		<0.001
Grade 1 varicocele	55 (23–76)		
Grade 2 varicocele	56 (18–78)		
Grade 3 varicocele	55 (12–75)		

Based on data from Ref. [19]
5–9 + 5 = 5–95th percentiles
The *p* values were obtained from regression analysis taking confounders into consideration
[a]Contains data from 6366 men because morphologic evaluation was not performed for all men
[+]Contains data from 6378 men because morphologic evaluation was not performed for all men

centration, sperm motility, and total motile sperm count, postoperatively. In addition to sperm parameters, varicocele repair probably has positive effects on Leydig cell function, improving serum testosterone level [22, 23]. Krishna Reddy et al. [24] showed total sperm motility of 30%, 29%, and 21% (World Health Organization lower reference limit [range]: 40 [38–42]) and mean sperm concentrations of 16.8 million/ml, 14.7 million/ml, and 9.75 million/ml (World Health Organization lower reference limit [range]: 15 [12–16]) in men presenting with infertility with grade 1, 2, and 3 varicocoele, respectively.

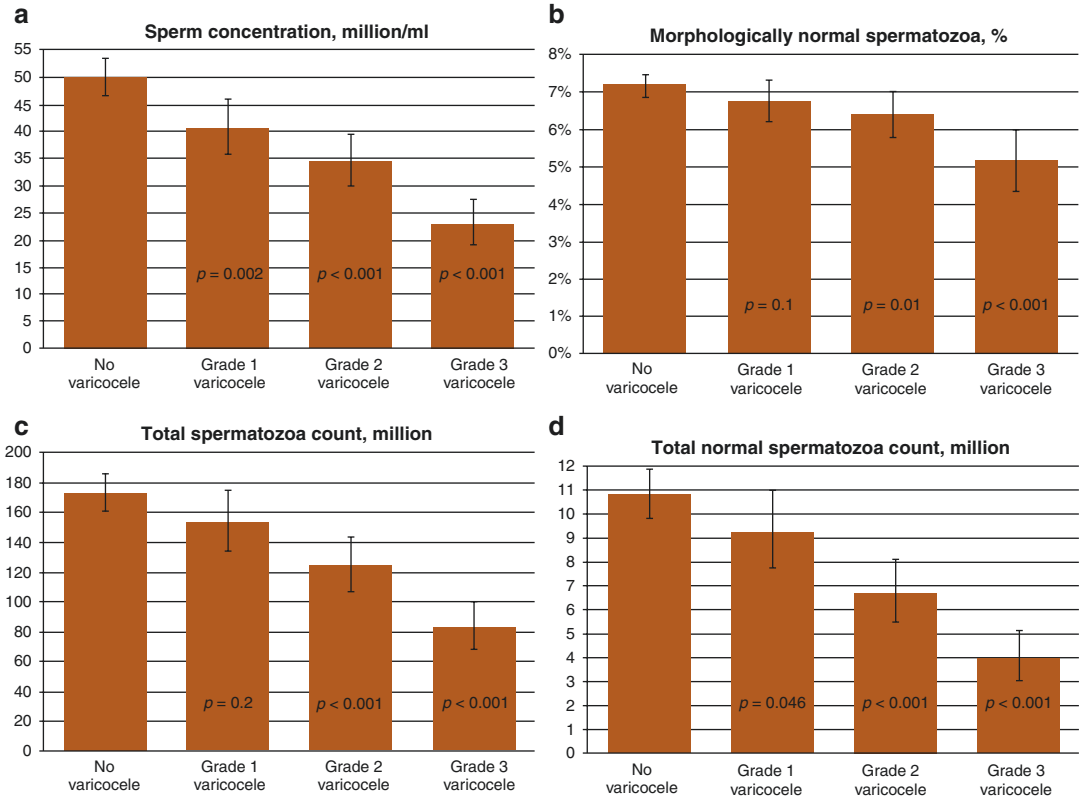

Fig. 21.1 Semen characteristics of 1102 men with varicocele classified as grade 1, 2, or 3 compared with 5933 unaffected men. Results are from the adjusted linear regression model. Error bars indicate 95% confidence interval. (**a**) Sperm concentration, (**b**) total spermatozoa count, (**c**) morphologically normal spermatozoa, and (**d**) total normal spermatozoa count. (Based on data from Ref. [2])

Table 21.3 Subgroup analyses

Subgroups	Number of studies (*K*)	Mean difference (95% CI)	I^2 (%)	Statistical model
Sperm count subgroup analysis				
WHO 2010	3	−51.21 (−76.14, −26.29)	97	REM
WHO 1999 and 1992	7	−41.04 (−69.36, −12.72)	98	REM
Fertile men	6	−65.00 (−73.98, −56.01)	92	REM
Normozoospermic men	4	−15.39 (−35.60, 4.83)	89	REM
Motility subgroup analysis				
WHO 2010	3	−34.39 (−48.37, −20.41)	98	REM
WHO 1999 and 1992	7	−23.56 (−32.55, −14.58)	96	REM
Fertile men	6	−32.03 (−41.58, −22.49)	96	REM
Normozoospermic men	4	−18.95 (−27.29, −10.61)	93	REM
Morphology subgroup				
WHO 2010	3	−21.38 (−43.17, 0.40)	100	REM
WHO 1999	5	−17.44 (−24.98, −9.90)	98	REM
Fertile men	4	−33.72 (−53.60, −13.84)	100	REM
Normozoospermic men	4	−5.87 (−9.57, −2.18)	96	REM

Based on date from Ref. [21]
WHO World Health Organization, *CI* confidence interval, *REM* random effects model

In the latest meta-analysis study done by Agarwal et al. (2016) [21], overall, 10 suitable studies—which included five cross-sectional, three cohort, and two case–control studies—were qualified for the study. These included 1232 men (783 with varicocele and 449 controls) [25–34]. The number of studies included in each meta-analysis varied according to the sperm parameter reported: six provided data on semen volume, 10 provided data on sperm count and motility, and eight provided data on morphology. All semen analyses were carried out following the WHO laboratory manual for the examination of human semen. Despite including only studies published after the release of the 2010 WHO manual (fifth edition) [12] only three of the studies specifically applied this new edition during semen analyses [30, 33, 34]. Six studies [25–31] utilized the previous version, namely the 1999 WHO manual (fourth edition), and one study [11] applied the 1992 WHO manual (third edition) for the analyses [10]. Of note, one of the studies that used the 1999 WHO manual utilized the strict criteria for sperm morphology assessment [11]. Most of the included studies were designed to evaluate the effect of varicocele on sperm functional parameters; semen characteristics as per the WHO laboratory manual were mainly secondary outcome measures. Six studies included fertile controls without varicocele and four studies included healthy normozoospermic controls without varicocele (with all semen parameters within normal ranges according to the WHO criteria utilized). They concluded that varicocele is a significant risk factor that negatively affects semen quality, but the observed pooled effect size on semen parameters does not seem to be affected by the WHO laboratory manual edition.

Semen Volume

In the meta-analysis by Agarwal et al. (2016) six studies' data on semen volume including 936 men (605 with varicocele and 331 controls) [26, 27, 29, 31, 33, 34] were analyzed. The mean semen volume in patients and controls ranged from 2.6 to 3.3 ml and 2.6 to 3.7 ml, respectively. In five studies, the data on semen volume were not statistically different between men with varicocele and controls [26, 27, 29, 31, 33]. Overall, REM indicated that semen volume was not significantly affected by varicocele (mean difference: [26, 27, 29, 31, 33] Overall, R 0.17; $P = 0.26$). The potential causes of the heterogeneity were analyzed by subgroup analysis to assess the effect of WHO manual editions for semen volume (Table 21.4) [21].

Table 21.4 Selection criteria of included studies (PICOS)

	Included	Excluded
Population	Infertile men (18 years of age and older) With clinical varicocele (any grade)	Azoospermia Subclinical varicocele Other risk factors for impaired semen quality Children and adolescents Fertile men with varicocele
Intervention	Semen analysis according to the WHO guidelines (any edition)	
Comparison	Men with proven or unproven fertility without clinical varicocele	
Outcomes	Semen volume (ml)	
	Sperm count ($\times 10^6$ ml^{-1})	
	Sperm motility (%)	
	Sperm morphology (%)	
Study type	Cross-sectional, case-control, and cohort studies published from 2010 onwards	

Based on data from Ref. [21]
WHO World Health Organization

Sperm Count

Madgar et al. [35] restricted their prospective study to men with sperm concentration between 5×10^6 and 20×10^6 ml^{-1} to limit the number of confounding variables, and they were able to demonstrate a significant improvement in sperm concentration, motility, and morphology (by 6 months postoperatively) and higher pregnancy rates than the control group. In another study Baazeem et al. [36] noted similar improvements in semen parameters in their recent review of 360 patients with sperm concentrations ranging 1×10^6–20×10^6 ml^{-1}. In the meta-analysis by Agarwal et al. [21] the included studies all reported the data on sperm count. Mean sperm count in patients and controls ranged from 9.62 to 96.6×10^6 ml^{-1} and 64.98 to 124.05×10^6 ml^{-1}, respectively. Eight studies reported a significant negative effect of varicocele on sperm count [37–44]. Overall, both FEM and REM indicated that sperm count significantly decreased in men with varicocele compared with controls. Given the high heterogeneity (98%), REM provides the most appropriate representation of the data. To analyze the potential causes of the heterogeneity, two subgroup analyses were conducted. First, the effect of WHO manual editions on sperm count estimates was assessed. The heterogeneity estimates and the observed pooled effect size were not materially affected by the WHO manual edition.

Sperm Motility

Asthenozoospermia is defined as sperm poor forward motility and represented as PR + NP motile sperms. According to Will et al. [45] 19% of subfertile men would suffer from asthenozoospermia if diagnosed with varicocele [45, 46]. The literature has consensus opinion that motility will improve in most of the patients where a palpable varicocele was treated [46–48].

In the meta-analysis by Agarwal et al. [21] the included studies all reported the data on sperm motility. Mean motility in patients and controls ranged from 21.1% to 61.9% and 49.3% to 70.0%, respectively. Nine studies reported a significant negative effect of varicocele on sperm motility [25, 27]. Overall, varicocele was a risk factor for motility and affected it significantly. As heterogeneity was high (97%), REM is more appropriate for estimating mean differences. To analyze the potential causes of the heterogeneity, two subgroup analyses were conducted according to the WHO manual edition and type of control. The heterogeneity estimates and the 167 observed pooled effect size were not materially affected by performing analyses separately by WHO manual editions. Like sperm count, the heterogeneity estimates were slightly reduced by performing analyses separately by type of controls (Table 21.4) [21]. The observed pooled effect size was larger for the studies using fertile controls compared with normozoospermic controls ($P = 0.04$). Sensitivity analyses indicated that the observed pooled effect size was not affected by removal of any of the studies. These results are, therefore, consistent in suggesting a negative association between varicocele and sperm motility.

Sperm Morphology

Most of the articles on sperm morphology are either retrospective or small studies. Hence controlled prospective studies are needed in getting more information. Few authors observed improvement in sperm morphology after varicocelectomy [48–50]. The study by Cakan et al. [50] showed no improvement in morphology and semen parameters in the control group with no pregnancies over a 12-month follow-up period. The meta-analysis by Agarwal et al. [51] had results with improvement in sperm morphology after treatment of the varicocele. In some studies in contrast to the above, a number of authors did not see any improvement in sperm morphology after varicocele repair [52, 53]. In the meta-analysis by Agarwal et al. (2016) [21] included eight studies, including 1092 subjects (713 with varicocele and 379 controls), were used in this analysis [25–27, 30–31]. In seven studies, varicocele was a risk factor for reduced sperm morphology [25–27, 30–31]. REM provided the most appropriate representation of the data since heterogeneity was high (100%). To analyze the potential causes of

the heterogeneity, two subgroup analyses were conducted (Table 21.4) [21] and, first, the effect of method for sperm morphology assessments was examined. Mean sperm morphology as per the WHO criteria (1999 edition) in patients and controls ranged from 8.4% to 30.8% and 21.2% to 72.0%, respectively. The results according to the strict criteria (2010 edition) in patients and controls ranged from 6.2% to 8.6% and 10.0% to 61.8%, respectively. Heterogeneity estimates were not affected by performing analyses separately according to the sperm morphology method. Similarly, the observed pooled effect size was not significantly affected by the sperm morphology method (WHO criteria: mean effect size with REM: −17.44%; 95% CI: −24.98%, −9.90%; and Strict criteria: mean effect size with REM: −21.38%; 95% CI: −43.17%–0.40%).

Time to Improvement

In a retrospective study by Al Bakri et al. [54] they evaluated the time taken to observe improvement in semen parameters. The authors concluded that after 3 months the maximum effect and benefit was observed [55]. There was a significant improvement in concentration and motility in the 100 men that met the inclusion criteria after 3 months, but this did not change at 6 months or longer. There were no statistically significant differences in the improvement of semen volume, motility, count, or total motile count among the results at 3, 6, and more than 9 months postoperatively.

Varicocele Repair—Consensus Recommendations

Varicocelectomy should be offered to the male partner in couples attempting to conceive only when all of the following conditions were present: a palpable varicocele, documented couple infertility, a female partner with normal fertility or potentially correctable infertility, and a male partner with one or more abnormal semen parameters or test results showing abnormal sperm function. The use of testicular volume as a predictor of fertility in patients with varicocele is still controversial [8, 56]. Two recent meta-analyses have shown that varicocelectomy significantly improves sperm concentration and motility in infertile men with palpable varicocele and abnormal preoperative semen parameters [57, 58]. Current evidence supports the idea that varicocele size does matter and that repair of larger varicoceles is more likely to improve seminal parameters than repair of smaller varicoceles [59]. Patients with higher sperm counts prior to repair had better results than those with more severe oligospermia [60]. The success of varicocele repair outcomes in infertile men was lower when there was the presence of Y chromosome micro-deletion, high follicle-stimulating hormone level, low testosterone level, significant testicular hypotrophy, and severe oligospermia [61]. There is no evidence of benefit from varicocele treatment in infertile men with normal results on semen analysis or in men with subclinical varicoceles [62]. The 2013 EAU guideline recommends varicocele treatment for adolescents presenting with palpable varicocele and ipsilateral testicular growth retardation. In adolescent males the semen analysis is an important additional indicator of the need for surgical intervention [63].

Treatment Options for Varicocele

The best treatment modality for varicocele can be chosen only after comparing the spontaneous pregnancy outcomes and complication rates of these approaches. Comparison of the seminal improvement after varicocele repair would not be unique among the techniques used for varicocele repair. The highest spontaneous pregnancy rate was seen with the microsurgical techniques. Recent meta-analyses suggested that a surgical varicocelectomy improved the spontaneous pregnancy rates for infertile men with low semen parameters and palpable varicoceles [58, 59]. Agarwal et al. [51] analyzed 17 studies reporting outcomes of microsurgical varicocelectomy and high ligation series for varicocele treatment in infertile men, and they demonstrated that surgical varicocelectomy significantly improves semen parameters in infertile men with palpable varicocele and abnormal semen analysis.

Evidence of Benefit of Varicocelectomy from Randomized Controlled Trials

Historical Evidence

In mid-twentieth century, Tulloch first reported improvement in semen parameters after high surgical ligation of varicose veins in 30 patients with infertility. The author showed a marked improvement in postoperative seminal parameters in 66% of the treated subjects [55]. Later different studies have supported Tulloch's initial findings. Okuyama et al. [64] conducted a case–control study on 224 men with clinical varicocele and subfertility by doing either varicocele repair by Palomo technique or expectant management. Follow-up of treated men for 1 year showed improvements in sperm density from $11.6 \times 106\ ml^{-1}$ to $25.3 \times 106\ ml^{-1}$ ($P < 0.01$) and percentage of progressive motile sperm from 21.3% to 30.2% ($P < 0.05$). Higher pregnancy rates in the group of treated men were also reported (30.6% Vs 18.1% in the control group, $P < 0.01$). Recently, Shamsa et al. [65] studied retrospectively 1711 patients who underwent microsurgical subinguinal repair for varicocele. Postoperatively, both sperm concentration (by 11.9%, $P < 0.001$) and sperm motility and morphology (38.3% to 41.1% and 54.5% to 56.5%, respectively, both $P < 0.01$) were found to be increased. In a study mentioned before, seven patients who were initially azoospermic showed sperms in their postoperative ejaculates. Al Bakri et al. [54] also documented improvement in sperm count from $18.2 \times 106\ ml^{-1}$ to $25.1 \times 106\ ml^{-1}$ 6 months after varicocele treatment ($P = 0.01$) in 100 men with varicocele confirmed by ultrasound examination and had subfertility.

Sperm Parameters Improvement—Evidence from Randomized Controlled Trials

A few randomized controlled trials (RCTs) as in Table 21.5 have examined the role of varicocele treatment on postoperative semen analysis. Mansour et al. [66] while evaluating 136 infertile couples, the male partners with clinical varicocele were randomly assigned to varicocelectomy or observation. The groups were matched according to female and male age, personal background, varicocele grade, and serum hormone values. The intervention group achieved significant improvements in semen parameters. Sperm concentration increased by 75%, whereas motility and morphology by 5.2% and 8%, respectively. In contrast, there were no obvious changes in semen parameters of control subjects within the same follow-up period of 12 months. Abdel et al. [67] analyzed 145 men randomly assigned for varicocele treatment or observation from a total of 150 men intended to treat. Patient characteristics were evenly distributed among groups, including varicocele grading. There was a significant improvement in sperm concentration ($18.1 \times 106\ ml^{-1}$ to $32.2 \times 106\ ml^{-1}$, $P < 0.01$), motility (25.3–41.0%, $P < 0.01$), and normal morphology (31.2–39.1%, $P < 0.01$) in the treatment group on a follow-up period of 12 months. No change was found on semen parameters during the follow-up period in the observation group.

Evidence from Meta-Analyses

Three meta-analyses done recently showed the benefits of varicocele repair with regard to semen parameters of subfertile men. Agarwal et al. (2007) [51] analyzed the 17 studies involving infertile men with clinical varicocele and with at least one abnormal semen parameter that are shown in Table 21.5. The treatment given was either high ligation of the veins or subinguinal microsurgical varicocelectomy. Microsurgical repair improved sperm concentration by $9.71 \times 106\ ml^{-1}$ and that with high ligation was by $12.03 \times 106\ ml^{-1}$. The sperm motility also increased after high ligation (by 9.92%) and microsurgical repair (by 11.72%). Sperm morphology change raised by 3.16% on the average of normal forms. Baazeem et al. [36] studied the role of varicocele repair on semen analysis in 22 different studies including meta-analyses, randomized and nonrandomized prospective controlled studies and analyzed sperm concentration

Table 21.5 Characteristics of included studies evaluating the effect of varicocele on semen parameters

First author, Year	Design	Patients and control	Patient (*n*)	Control (*n*)	WHO semen parameters evaluated	WHO edition
Abd-Elmoaty (2010)	Cross-section	Infertile men with clinical varicocele (any grade) and fertile men without clinical varicocele[a]	32	18	Sperm count, sperm motility, and sperm morphology	1999
Blumer (2011)	Cross-section	Infertile men with clinical varicocele (grades 2 and 3) and normozoospermic[b] men without clinical varicocele	30	32	Semen volume, sperm motility, progressive motility total sperm count, sperm morphology, total motile, and morphologically normal sperm count	1999[f]
Camejo (2011)	Cross-section	Infertile men with clinical varicocele (grades 2 and 3) and normozoospermic[b] men without clinical varicocele	67	44	Semen volume, sperm density, progressive motility, sperm morphology, and sperm vitality	1999
Chan (2013)	Case-control	Infertile men with clinical varicocele (grade 2) and fertile semen donors without clinical varicocele[c]	20	20	Sperm density and sperm motility	1999
Mohamed (2011)	Case-control	Infertile men with clinical varicocele (grade 2 and 3) and fertile[d] men without clinical varicocele	50	50	Semen volume, sperm density, and sperm motility	1992
Tawadrous (2013)	Cross-section	Infertile men with clinical varicocele (any grade) and fertile[e] men without clinical varicocele	54	60	Sperm density, sperm motility, and sperm morphology	2010
Vivas-Acevedo (2010)	Cross-section	Infertile men with clinical (any grade) and normozoospermic[b] men without clinical varicocele	352	155	Semen volume, sperm density, progressive motility, sperm vitality	1999
Sadek (2011)	Cohort; prospective	Infertile men with clinical varicocele (any grade; left side) and fertile[e] men without clinical varicocele	72	20	Sperm density, sperm motility, and sperm morphology	1999
Mostafa (2014)	Cohort; prospective	Infertile men with clinical varicocele (any grade) and fertile[e] men without clinical varicocele	46	20	Semen volume, sperm density, sperm motility, and sperm morphology	2010
Vivas-Acevedo (2014)	Cohort; prospective	Infertile men with clinical varicocele (grade 2 and 3) and normozoospermic[b] men without clinical varicocele	60	30	Semen volume, sperm density, sperm motility, sperm morphology, and sperm vitality	2010

Based on data from Ref. [21]
WHO World Health Organization
[a]Definition of fertility not included
[b]Fertility status not stated
[c]Had fathered a child in the past 3 years
[d]Fertility was confirmed by history of at least 1 offspring
[e]Men who initiated at least 1 natural pregnancy in the previous year
[f]Morphology assessed by strict criteria

before and after repair of clinical varicoceles in men with infertility and abnormal semen parameters. They found an increase in sperm concentration of 12.32×10^6 ml^{-1} from before to after interventions. In their study, sperm motility data were available from 17 prospective studies. Using the random effects model, a combined increment of 10.86% in motility was demonstrated. They also evaluated five prospective studies reporting the percentage of progressive motile sperm and confirmed significant improvement in progressive sperm motility of 9.69% using the random effects model. Finally, Schauer et al. [68] in their meta-analysis studied the impact of each type of intervention; namely, high ligation, inguinal varicocelectomy, and subinguinal varicocelectomy on the semen parameters of subfertile men by combining 14 studies including randomized controlled trials, interventional trials, and cohort studies totaling 1476 subjects. Inclusion criteria included subfertility and/or at least one abnormal semen parameter, clinical varicocele, and 19 years of age or older. There was a significant improvement in sperm count (by 10.85×10^6 ml^{-1} on average; $P = 0.006$) and motility (by 6.80% on average; $P < 0.001$) with all techniques studied with minimal differences between intervention groups. The increase in sperm count and sperm motility achieved by inguinal approaches was of no clinical significance when compared to other techniques. This meta-analysis indicated that varicocelectomy results in significant sperm count and motility with any chosen surgical technique. All these studies showed improved semen parameters of infertile men with clinical varicocele [69].

Conclusions

Clinically detected varicocele was found to be a significant risk factor for decreased sperm count, motility, and morphology in adult infertile men. Significant improvement in sperm concentration as well as total and progressive sperm motility was seen after varicocele repair as reported in several studies. The observed pooled effect size does not seem to be affected by the WHO laboratory manual edition used for the examination of human semen. Given most of the studies published after 2010 still utilized the 1999 manual for semen analysis, further research is required to fully understand the impact of this change on the association between varicocele and semen parameters.

Review Criteria

We conducted an electronic search using search engines such as Science Direct, Scopus, OVID, Google Scholar, PubMed, and MEDLINE databases until 2017 to investigate the current status of varicocele repair. There were no limits placed on the year of publication, but we restricted the search to articles published in English. The search utilized the keywords "Varicocele", "Varicocele repair", "Male infertility", "Review", and "Semen parameter". The findings of a recent meta-analysis on varicocele repair were reviewed and so were the current status of varicocele repair for infertile men and the effects on semen parameters and male infertility.

Multiple Choice Questions and Answers

1. What modality is used most often to diagnose a varicocele?
   (a) Pelvic CT
   (b) Scrotal MRI
   (c) **Scrotal US**
   (d) Testicular scintigraphy
2. Which of the following about varicoceles is FALSE?
   (a) **More common in the right scrotum**
   (b) Results from the dilatation of the pampiniform venous plexus

(c) Feels like a "bag of worms"
(d) The swelling is painless

3. During surgery, you observe that the left renal vein is engorged but the left kidney is normal. What should next be examined?
   (a) Right renal vein
   (b) **Inferior vena cava**
   (c) Right testicular vein
   (d) Left testicular vein
4. Which of the following statements about varicoceles is FALSE?
   (a) **It commonly occurs on the right side**
   (b) It results from the dilatation of the pampiniform venous plexus
   (c) Its typical "bag of worms" appearance on palpation makes its diagnosis apparent
   (d) It commonly causes a non-tender swelling
5. How does a varicocele affect the semen analysis?
   (a) **Decreased sperm count with an increase in the number of abnormal forms**
   (b) Increased sperm count with absent motility
   (c) Decreased sperm count with an increase in motility
   (d) Azoospermia
6. Which of the following is the most common cause of male infertility?
   (a) Testicular failure
   (b) Obstruction
   (c) Low semen volume
   (d) **Varicocele**
7. What is not true about varicoceles?
   (a) They are ten times more likely to occur on the left than on the right
   (b) Varicoceles are present in about 15% of the male population
   (c) Varicoceles are present in 25% to 40% of males with male factor infertility
   (d) **Varicoceles are thought to influence semen quality by decreasing testicular temperature**
8. Which method of varicocele repair has the highest risk of complication of arterial injury and thrombophlebitis?
   (a) Retroperitoneal ligation of the internal spermatic vein
   (b) Inguinal ligation
   (c) Infra inguinal ligation
   (d) **Transfemoral vein embolization by interventional radiology**
9. What is the most common complication of varicocele repair?
   (a) **Hydrocele formation**
   (b) Testicular hypertrophy
   (c) Hemorrhage
   (d) Spermatocele
10. What is a true statement about isolated right-sided varicoceles?
    (a) They compress easily
    (b) They are seen more commonly than varicoceles on the left
    (c) **Presence demands assessment of kidney and renal hilar regions for a possible mass**
    (d) They do not typically cause infertility
11. Which of the following is an enlargement of the veins of the spermatic cord that commonly causes a "bag of worms" exam in the affected hemi scrotum?
    (a) A spermatocele
    (b) A hydrocele
    (c) Capistation
    (d) **A varicocele**
12. What is the most common identifiable cause of male infertility?
    (a) Antisperm antibodies
    (b) Azoospermia
    (c) **Varicocele**
    (d) Sperm motility disorders
13. A 15-year-old male presents for a sports physical. He is sexually active and states that he uses condoms. He denies dysuria or urethral discharge. There is an area of swelling above the right testicle that feels like a bag of worms. It is not tender and the testicles are smooth. What is the most appropriate management?
    (a) Testing for gonorrhea and chlamydia
    (b) Reassurance
    (c) **Doppler ultrasound**
    (d) Referral for surgery
14. Which is often associated with a varicocele of the spermatic cord?
    (a) Trauma
    (b) Sexually transmitted infections

(c) **Infertility**
(d) Impotence

15. What is significant about a varicocele scrotal mass?
    (a) Can be transilluminated
    (b) Usually occurs on the right side
    (c) **Is less apparent in the supine position**
    (d) Commonly occurs bilaterally
16. You are evaluating a male for infertility. You decide to send him for an ultrasound to determine the presence of a varicocele. On sonogram, at what spermatic vein diameter is the diagnosis of a varicocele made?
    (a) Greater than 1 mm
    (b) **Greater than 3 mm**
    (c) Greater than 7 mm
    (d) Greater than 10 mm
17. A 50-year-old obese male presents to the clinic with "lumpiness" on the right side of his scrotum. He denies any pain and has not had any fever, urethral discharge, or dysuria. The patient has noticed an unintentional 15 lb weight loss in the last 3 months. The patient has a history of alcohol use disorder and has smoked one pack of cigarettes for 30 years. Upon physical examination, there is a non-tender tortuous mass within the right scrotum that does not transilluminate. What about the patient's history is most concerning for existing malignance?
    (a) Non-tender mass that does not transilluminate
    (b) Obesity
    (c) **The side of scrotum involved**
    (d) History of alcoholism and tobacco abuse

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Effect of Varicocele Treatment on Oxidative Stress Markers and Sperm DNA Fragmentation

22

Bryan Naelitz and Neel Parekh

Key Points

- Varicocele is a condition characterized by incompetent veins of the pampiniform plexus that impair venous drainage and permit reflux, which induces heat stress and relative testicular hypoxia.
- Heat stress, perturbations in cellular metabolism, and disruption of the electron transport chain result in production of reactive oxygen species that damage the testes and impair spermiogenesis.
- Varicocele repair attenuates oxidative stress, as evidenced by normalization of biomarkers measuring lipid peroxidation, protein modification, and DNA fragmentation.
- Varicocelectomy should be offered to infertile men with clinical varicocele and abnormal semen parameters. Markers of oxidative stress and sperm DNA fragmentation will improve following repair in most individuals.
- Antioxidant therapy is inexpensive and improves markers of oxidative stress and sperm DNA fragmentation. Antioxidants are not alternatives to varicocelectomy, but are being investigated as an adjuvant therapy to surgical repair.

Introduction to Oxidative Stress and Mechanisms of Oxidative Damage

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are labile compounds generated during metabolic reactions that serve critical roles in a variety of cellular processes [1]. Reactive chemical species are typically classified into two categories: free radicals and non-radical groups. Free radicals are highly reactive, short-lived compounds that carry an unpaired electron [2]. Given their instability, free radicals abstract electrons from neighboring compounds, which, in turn, become radicalized and perpetuate a chain of electron transfers that oxidize biomolecules, altering their chemical structure [3]. In contrast, non-radical groups are relatively stable chemical species with fully paired valence electrons that possess strong oxidizing potential [3]. The oxidative potential generated by these species is balanced by antioxidants, which accept electrons from chemical groups in high energy

B. Naelitz
Glickman Urological and Kidney Institute, Cleveland Clinic Lerner College of Medicine, Cleveland, OH, USA

N. Parekh (✉)
Glickman Urologic and Kidney Institute, Cleveland Clinic, Cleveland, OH, USA
e-mail: parekhn3@ccf.org

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states [4]. This reduction reaction neutralizes the chemical cascade that rapidly generates ROS and RNS, curbing oxidative activity [5].

Reactive chemical species are essential for the activation of sperm and the subsequent processes of capacitation, the acrosome reaction, and oocyte fusion [6]. However, overproduction of reactive species or reduced antioxidant capacity generates oxidative stress that damages cells. Excessive oxidative potential affects all biomolecules, but has pronounced effects on the polyunsaturated fatty acids comprising cellular membranes [7, 8]. Oxidative stress also damages DNA by chemically modifying nitrogenous bases and disrupting the sugar-phosphate backbone. Such derangements are associated with DNA fragmentation and loss of genetic integrity [9]. These sequelae are especially detrimental to sperm because transmission of functional genetic material is essential for successful fertilization and normal embryonic development [10].

In the chapter that follows, we will explore the mechanisms by which varicocele generates reactive chemical species and review the effects of medical and surgical treatment on markers of oxidative stress and DNA fragmentation. Controversies surrounding varicocele repair and the future of varicocele management will also be explored.

Pathophysiology of Varicocele-Induced Oxidative Stress and Sperm DNA Fragmentation

Varicocele is an "abnormal dilatation, elongation, and tortuosity of the pampiniform plexus veins of the spermatic cord" that results in poor blood flow through the testis, affecting the left side more commonly than the right [11]. Though several mechanisms have been proposed to explain how varicoceles impair male fertility, the most widely accepted theory is that relative stasis of venous blood in the pampiniform plexus increases testicular temperature, resulting in metabolic changes that increase the burden of oxidative stress on the testes [12].

Multiple controlled trials have demonstrated that infertile men with varicocele produce semen with higher levels of reactive species than men with preserved fertility [13, 14]. Some of these studies demonstrated that free radicals such as nitric oxide and non-radical groups like hydrogen peroxide were elevated in the semen of infertile men with varicocele when compared to fertile controls [15–18]. Other studies illustrated that indirect markers of oxidative stress, including malondialdehyde and hexanoyl-lysine, were higher in the semen of men with varicocele [14, 19]. Evidence of oxidative stress was also appreciated in testicular tissue: both direct and indirect markers of oxidative stress were found to be elevated in testicular biopsies taken at the time varicocele repair surgery [20]. Taken together, these findings support the hypothesis that varicocele mediates a pathological process that generates higher levels of oxidative stress along the male reproductive tract.

Though oxidizing reactions are necessary for sperm activation, excessive oxidative stress observed in the setting of varicocele is detrimental for sperm maturation. Spermatogenesis optimally occurs at temperatures 2 °C to 3 °C below core body temperature [21]. However, the irregularly dilated veins of varicocele result in poor drainage from the pampiniform complex, which results in stasis of venous blood and elevations in scrotal temperature [22, 23]. Evidence that heat stress negatively impacts semen quality comes from animal models that demonstrated a temperature-dependent relationship between elevated scrotal temperatures and indirect markers of oxidative stress in semen [24]. Some testicular cells—spermatogonia A, Leydig cells, Sertoli cells—are generally tolerant of temperature increases and the resulting burden of oxidative stress. Others are not: spermatogonia B, spermatids, and spermatocytes in the pachytene stage of prophase are especially vulnerable to heat stress and deteriorate with prolonged exposure [25]. The resulting cellular damage reduces male fertility not only by impairing sperm production, but also by altering the motility and morphology of the surviving gametes [26].

Oxidative stress alters the chemical structure of DNA in a variety of ways, the most significant of which is the molecular fragmentation that

results from strand breaks [27]. DNA fragmentation occurs throughout the male reproductive tract, but affects sperm in the excurrent duct system to a greater extent than developing gametes located in the testes [28, 29]. Reactive chemical species are believed to be the major cause of DNA fragmentation with many studies demonstrating a strong correlation between oxidative stress and DNA damage [30, 31]. One such study by Henkel and colleagues revealed that increasing levels of seminal ROS were associated with greater measures of sperm DNA fragmentation [32]. In addition to reflecting spermatic exposure to oxidative stress, DNA fragmentation is an important metric because it directly reflects the integrity of the genetic material carried by these gametes [33]. Though the clinical significance of DNA fragmentation is still being investigated, elevated sperm DNA fragmentation indices have been associated with lower pregnancy rates with intrauterine insemination, increased rates of aneuploidy, and worse outcomes with intracytoplasmic sperm injection [34, 35].

Given that varicocele increases the seminal burden of oxidative stress, it is unsurprising that clinical studies have demonstrated an association between this condition and DNA damage [36]. For example, a recent cross-sectional study by Dieamant and colleagues analyzed 2399 infertile men who underwent semen analysis. The 391 men with clinical varicocele were found to produce sperm with a greater degree of DNA fragmentation than the 2008 men without varicocele (16.3% vs. 15.3%, $P = 0.03$) [37]. Other studies have compared sperm DNA fragmentation outcomes between infertile men with varicocele to normozoospermic controls. Smith et al. showed that 49% of varicocele patients with normal semen parameters and 58% of those with abnormal semen profiles had sperm DNA fragmentation values two standard deviations or greater when compared to normozoospermic controls [38].

The association between varicocele and elevated sperm DNA fragmentation values has been further substantiated by systematic reviews. One report by Zini and Dohle assessed case–control studies to clarify how fertility and varicocele status affects sperm DNA fragmentation rates [39]. The authors identified nine studies that compared sperm DNA fragmentation values between infertile men with varicocele and those without. Four of these studies found rates of sperm DNA fragmentation to be higher in the varicocele cohorts. Additionally, six of seven case-control studies demonstrated that fertile studies demonstrated that fertile men with varicocele had higher levels of sperm DNA fragmentation than those without the diagnosis. A meta-analysis by Wang and colleagues evaluated seven studies that compared 240 men with varicocele to 176 controls without the condition [40]. Sperm DNA fragmentation was found to be higher in men with varicocele than those in the control group, with a mean difference of 9.84% (CI: 9.19 to 10.49%, $P < 0.0001$).

The available evidence suggests a relationship between clinical varicoceles, elevated markers of oxidative stress, and higher rates of sperm DNA fragmentation. Additional research is required to clarify the connection between these outcome measures to better understand the impact of varicocele-induced oxidative stress on male fertility.

Overview of Varicocele Treatment

There are two options to consider for the definitive treatment of varicocele: surgery and percutaneous embolization. Both of these interventions share the goal of obstructing the internal spermatic veins to prevent reflux of venous blood [41]. Repair is performed with the intention of preventing further damage to the gonad with the hope of enhancing spermatogenesis [42]. Though male infertility associated with palpable varicocele has traditionally been managed with surgery, embolization, or assisted reproductive technology, there has been increased interest in medical therapy given strong evidence that oxidative stress is responsible for testicular damage [43]. Antioxidants are electron-accepting compounds that have the capacity to neutralize reactive chemical species and reduce the oxidative stress burden in reproductive tissues. Accordingly, agents such as ascorbic acid, tocopherol, carnitine, zinc, and folic acid have been trialed as potential treatments

for varicocele-induced infertility [44]. Non-steroidal anti-inflammatory drugs (NSAIDs) have also been proposed for treating subfertility in the setting of varicocele [44]. NSAIDs inhibit production of prostaglandin and leukotriene intermediates in the canonical inflammation cascade mediated by immune effectors. As a result, leukocyte activation is curbed, reducing the production of reactive oxygen species [45].

Effect of Varicocelectomy on Markers of Oxidative Stress

There is moderate evidence supporting the efficacy of varicocele repair in correcting seminal and peripheral markers of oxidative stress. Prior to intervention, varicocele increases the burden of oxidative stress on the male reproductive tract secondary to increased heat stress, hypoxia, and mitochondrial dysfunction that result from poor venous outflow [46]. Varicocelectomy corrects this underlying pathology and has been shown to reduce seminal markers of lipid peroxidation, reactive oxygen species, and reactive nitrogen species in the majority of clinical studies [16, 47, 48]. However, not all research has demonstrated that markers of oxidative stress improve following surgical intervention. A controlled study by Yesilli and colleagues failed to reveal statistically significant differences in seminal malondialdehyde levels 6 months following varicocele repair ($P = 0.65$), despite showing increases in enzymatic markers of sperm maturation [49]. Other studies reaching a similar conclusion included younger patients and those without a diagnosis of infertility [50, 51]. Inclusion of these patients in study analyses has invited criticism, since young, fertile patients are more likely to posses normal levels of oxidative stress markers prior to surgery. Thus, the power of these studies to assess postoperative changes in men experiencing an increased burden of seminal oxidative stress may be reduced [52]. Careful selection of study population is necessary to limit study heterogeneity and ensure the trial has the capacity to evaluate how specific populations may benefit from varicocele repair. Table 22.1 summarizes the findings of the studies noted above.

Table 22.1 Studies evaluating the effect of varicocele and varicocelectomy on markers of oxidative stress

Study	Design	Patients	Controls	Key findings
Sakamoto et al. [16]	Retrospective cohort	15 oligozoospermic and 15 normozoospermic men with varicocele	15 oligozoospermic and normozoospermic men without varicocele	Varicocelized men undergoing microsurgical varicocelectomy experienced statistically significant decreases in seminal levels of nitrous oxide (17.1 vs. 7.5 umol/L, $P < 0.001$), 8-OHdG (10.3 vs. 6.2 umol/L, $P < 0.001$), hexanoyl-lysine (137.3 vs. 90.9 umol/L, $P = 0.005$), and superoxide dismutase activity (85.8 vs. 78.1%, $P = 0.01$)
Hurtado de Catalfo et al. [47]	Prospective cohort	36 infertile men with unilateral left varicocele	33 age-matched, proven-fertile men	Men undergoing varicocele repair experienced a reduction in seminal and peripheral TBARS assay activity when assessed 1–3 months after surgery ($P < 0.001$). Protein carbonyl levels normalized in the same time period. Seminal levels of nitric oxide synthase activity were reduced after 3 months of recovery ($P < 0.001$), but remained unchanged in the peripheral circulation

Table 22.1 (continued)

Study	Design	Patients	Controls	Key findings
Mostafa et al. [48]	Prospective cohort	68 infertile males scheduled for varicocelectomy	None	Study participants experienced a significant decline in level of malondialdehyde (23.2 vs. 15.3 nm/mL, $P < 0.0001$), hydrogen peroxide (45.2 vs. 39.4 nm/mL, $P < 0.0001$), and nitric oxide (5.8 vs. 5.5 nm, $P = 0.0002$) 3 months after varicocelectomy. Levels of malondialdehyde (15.3 vs. 12.2 nm/mL, $P < 0.0001$), hydrogen peroxide (39.4 vs. 34.8 um/mL, $P < 0.0001$), and nitric oxide (5.5 vs. 5.0 nm/L, $P = 0.0014$) continued to improve 6 months following intervention
Yesilli et al. [49]	Not indicated	56 infertile men with varicocele	25 healthy, normospermic donors	The study did not find a statistically significant difference in measures of seminal malondialdehyde levels 6 months after microsurgical varicocelectomy (0.61 vs 0.58 nmol/10^9 sperm, $P = 0.65$). However, activities of sperm HSPA2 increased following the intervention (3.15 vs. 8.56 IU/10^8 sperm, $P < 0.001$)
Rodriguez Pena et al. [50]	Not indicated	202 men with left grade II or grade III varicocele referred for testicular pain or for employee health examination	None	Seminal levels of nitric oxide were unchanged 6 months after varicocelectomy and did not correlate with sperm motility ($P = 0.89$), concentration ($P = 0.89$), varicocele grade ($P = 0.70$), or testicular volume ($P = 0.47$)
Lacerda et al. [51]	Prospective cohort	27 adolescents with grade II or grade III varicocele	None	No statistically significant differences were observed regarding seminal malondialdehyde concentration 3 months following varicocele repair (307.6 vs 317.6 ng/mL, $P = 0.93$)
Chen et al. [53]	Prospective cohort	30 infertile males with varicocele confirmed with Doppler ultrasound	None	Varicocelectomy improved seminal levels of 8-OHdG, a marker of DNA oxidation, (10.27 vs. 5.95 molecules/10^5 dG, $P = 0.012$) and increased the concentration of ascorbic acid in the ejaculate (1.87 vs. 3.12 mg/dL, $P = 0.012$)
Shiraishi et al. [57]	Prospective cohort	37 infertile patients with left varicocele confirmed with physical examination and Doppler ultrasound	Semen from 8 patients with obstructive azoospermia with normal spermatogenesis	Patients who responded to varicocele repair, as indicated by a statistically significant improvement in motile sperm count, had higher preoperative levels of 4-HNE modified proteins, markers of lipid peroxidation and oxidative stress, at the time of surgery than non-responders (247.9 vs. 152.6% of control values, $P = 0.14$)

8-OHdG 8-hydroxy-2′-deoxyguanosine, *TBARS* thiobarbituric acid reactive substances, *HSPA2* heat shock protein family A (Hsp70) member 2, *4-HNE* 4-hydroxy-2-nonenal

In addition to attenuating production of reactive species, varicocele repair has been shown to improve the total antioxidant capacity of blood plasma and the concentration of antioxidants (including retinol, ascorbate, zinc, selenium, glutathione, and albumin) in semen [47, 53–55]. The effect on seminal levels of vitamin E is less clear with some studies showing improvement following varicocele repair and others showing a reduction [47, 48]. Vitamin E is an essential nutrient, meaning that levels measured in blood and semen are highly dependent on dietary intake [52]. This variability must be controlled for in studies assessing the impact of surgical intervention on concentrations of this micronutrient. There is similar uncertainty surrounding the effect of varicocele repair on the expression of enzymatic antioxidants including glutathione peroxidase, superoxide dismutase, and catalase. A study performed by Mostafa and colleagues revealed that expression of these enzymes was increased 3 and 6 months after varicocele repair [48]. However, other research has concluded that enzymatic antioxidant activity—elevated in the setting of varicocele—declines following surgery [47]. Though additional research is necessary to elucidate the full spectrum of antioxidant effects resulting from varicocelectomy, the available evidence indicates that oxidative stress generally declines following surgical intervention.

Interestingly, measuring markers of oxidative stress may enhance candidate selection for varicocele repair. This idea stems from the observation that elevations in oxidative stress markers predict improvements in semen parameters following surgery [56]. A study by Shiraishi and colleagues demonstrated that men with elevated preoperative levels of 4-hydroxynonenal-modified proteins, markers of lipid peroxidation and oxidative stress, experienced greater improvements in sperm count, motility, and morphology following surgery ($P = 0.014$) [57]. Other research has indicated that normalization of oxidative stress markers following varicocelectomy is associated with improved sperm quality [47, 48]. These observations are consistent with the current understanding of varicocele pathophysiology: selecting patients with high levels of seminal oxidative stress for surgery may increase benefit because correcting this condition has the potential to normalize the oxidative milieu. However, not all patients experience improvements in fertility following varicocele repair [58]. The reason for a poor response to varicocelectomy is unclear, but some have suggested that some men possess a greater capacity to buffer against oxidative stress and that reducing the burden of reactive chemical species may provide less benefit to these patients [52]. Addressing this question remains an active area of research.

Effect of Varicocelectomy on Sperm DNA Fragmentation

Varicocele repair has long been performed to improve fertility outcomes, but has more recently been used to improve the integrity of sperm DNA via reduction of oxidative stressors. It is important to understand that the effects of varicocelectomy are time-dependent and that laboratory studies may not reflect the full extent of improvement until several months following surgical intervention [59].

There is substantial evidence that varicocelectomy improves the integrity of sperm DNA as indicated by measures of DNA fragmentation. A recent review article by Roque and Esteves examined 20 studies that assessed this outcome variable [60]. These studies encompassed over 1200 patients, nearly all of whom possessed a clinically palpable varicocele and abnormal semen measures. Incredibly, all of these studies demonstrated an improvement in sperm DNA fragmentation following surgical intervention, despite immense heterogeneity with respect to study design, sample size, follow-up time, and type of DNA fragmentation assay employed.

Some studies compared the effects of varicocelectomy among patients without employing a control group. For example, Zini and colleagues evaluated the effect of microsurgical varicocele repair in patients with palpable varicocele and found that the DNA fragmentation index improved following surgery (18% vs. 10%, $P < 0.001$) [61]. A larger study performed by Smit et al. assessed the same parameter in men with clinical varicocele and infertility and again demonstrated a statistically significant reduction

in the DNA fragmentation index following the procedure (35% vs. 30%, $P = 0.02$) [62]. The results of these studies are consistent with those of a meta-analysis that included 177 patients with varicocele from six studies that reported a mean improvement of 3.4% in sperm DNA fragmentation with varicocelectomy [40]. Table 22.2 summarizes the findings of these studies and other trials examining the impact of varicocele repair on sperm DNA fragmentation.

Other studies employed control groups to compare the impacts of surgical intervention on postoperative markers of DNA integrity between fertile and infertile populations. For example, Li and colleagues compared 19 infertile men with clinical varicocele to an equal number normozoospermic controls and found that sperm DNA fragmentation was higher among infertile participants prior to surgery (28.4% vs. 17.4%, $P = 0.007$) [63]. This study found that sperm DNA fragmentation decreased postoperatively among infertile men with varicocele (28.4% vs. 22.4%, $P = 0.018$) and that postoperative fragmentation rates in varicocele patients were similar to the fertile controls. Similarly, La Vignera and colleagues demonstrated that microsurgical

Table 22.2 Studies evaluating the effect of clinical varicocele and varicocelectomy on sperm DNA fragmentation

Study	Design	Patients	Controls	Key findings
Zini et al. [61]	Prospective cohort	25 men with varicocele and abnormal semen parameters	None	SDF improved from preoperative baseline (18 ± 11%) when assessed at 4 months (10 ± 5%, $P = 0.0009$) and 6 months (7 ± 3%, $P = 0.0021$) after microsurgical repair
Smit et al. [62]	Prospective cohort	49 men with varicocele and oligozoospermia	None	SDF improved following treatment with either high inguinal ligation or microsurgical repair with mean preoperative SDF falling from 35.2% to 30.2% in the postoperative period ($P = 0.019$)
Wang et al. [40]	Meta-analysis	240 men with varicocele	176 healthy donors	SDF is higher in men with clinical varicocele than healthy controls, with a mean difference in SDF indices of 9.84% (95% CI: 9.19–10.49%, $P < 0.00001$)
Li et al. [63]	Not indicated	19 infertile men with varicocele	19 normozoospermic donors	SDF was higher in varicocelized men than in the normozoospermic controls prior to repair (28.4 vs. 17.4%, $P = 0.007$). Microsurgical varicocelectomy reduced SDF in the varicocele cohort (18.4 vs 22.4%, $P = 0.018$)
La Vignera et al. [64]	Not indicated	30 oligoasthenoteratozoospermic men with left, grade 3 varicocele	30 normozoospermic donors	SDF improved from preoperative baseline (5.0 ± 3.0%) when assessed 4 months (2.1 ± 0.4%, $P < 0.05$) following microsurgical repair. The percentage of sperm with low mitochondrial membrane potential (2.0 vs. 28.0%, $P < 0.05$), phosphatidylserine externalization (3.0% vs 9.0%, $P < 0.05$), and chromatin decondensation (6.0% vs. 22.0%, $P < 0.05$) improved following intervention
Ni et al. [65]	Prospective cohort	42 men with various grades of left-sided varicocele and abnormal semen parameters	10 normozoospermic donors	DFI measures among men with varicocele were higher in the varicocele cohort prior to microsurgical repair than in the control group (27.4 vs. 11.5%, $P < 0.01$). Among couples achieving pregnancy, DFI values improved following varicocelectomy (27.4 vs. 20.6%). DFI was significantly less in couples achieving pregnancy than in couples failing to do so (20.6 vs. 24.7%, $P < 0.01$)

SDF sperm DNA fragmentation, *DFI* DNA fragmentation index

varicocelectomy on men with oligoasthenoteratozoospermia and grade 3 varicocele improves sperm DNA fragmentation rates (5.0% vs. 2.1%, $P < 0.05$), and that postoperative values approach those of the nomozoospermic controls (2.1% vs. 2.0%) [64]. Interestingly, this study concomitantly examined the effects of surgical repair on markers of oxidative stress, including mitochondrial membrane potential, phosphatidylserine externalization, and chromatin compactness. All markers of oxidative stress declined following varicocelectomy and did not differ in a statistically significant fashion from the control group. These studies reinforce the idea that varicocele repair not only improves measures of oxidative stress and DNA damage, but that these values normalize following surgery.

Postoperative sperm DNA fragmentation rates may correlate with pregnancy success as demonstrated in the few studies. Smit and colleagues prospectively followed 49 men with palpable varicocele, oligozoospermia, and infertility who underwent surgical repair [62]. Couples that achieved a pregnancy naturally or with ART after varicocele repair had lower sperm DNA fragmentation rates compared to those who were unsuccessful (26.6% vs. 37.3%, $P = 0.013$). These results were echoed in a study by Ni et al. that compared men with high-grade varicocele repair to a control group of semen donors [65]. This study found that sperm DNA fragmentation decreased following the intervention (28.4% vs. 22.4%, $P = 0.018$) and that male partners in couples that achieved pregnancy had significantly lower markers of DNA damage compared to those who were unsuccessful (20.6% vs. 24.7%, $P < 0.01$). Though further research must be performed to evaluate the clinical utility of sperm DNA fragmentation, a reduction of this metric may be predictive of postoperative improvement in male fertility.

Antioxidant Therapy as Treatment for Oxidative Stress

Antioxidants have been proposed as a potential treatment for varicocele-induced infertility given their potential to decrease oxidative stress by neutralizing reactive chemical species that medicate testicular damage and derangements in spermiogenesis [66]. These agents have elicited attention from investigators because they offer a noninvasive approach for addressing subfertility, are relatively inexpensive, and generally possess limited side effect profiles [44]. A variety of antioxidant monotherapies and combination therapies have been studied in the past two decades. A selection of studies assessing the impact of therapy on markers of oxidative stress and DNA fragmentation is discussed below.

Vitamin E possesses strong antioxidant properties and can directly neutralize reactive chemical species including hydroxyl radical and superoxide anions. This fat-soluble compound may also have anti-inflammatory activity and may theoretically reduce leukocyte-induced oxidative stress on the male reproductive tract [67]. Two studies have examined the impact of vitamin E monotherapy on sperm malondialdehyde, a marker of lipid peroxidation and seminal oxidative stress. The first of these by Geva and colleagues found that supplementation with 200 mg of vitamin E per diem significantly reduced malondialdehyde levels within 4 weeks' time [68]. The second study by Suleiman et al. reached the same conclusion after showing that daily administration of 300 mg of vitamin E reduced seminal levels of malondialdehyde [69]. Some studies have examined the effect of vitamin C and E combination therapy on markers of oxidative stress. Vitamin C is an essential nutrient found in high quantities in semen that neutralizes reactive chemical species and augments vitamin E recycling [70, 71]. A placebo-controlled randomized controlled trial by Greco et al. addressed this question by administering 1000 mg of both vitamins for 2 months, noting a significant decrease in the percentage of spermatozoa positive for DNA fragmentation in the subgroup of men with semen parameters that improved in response to therapy (24.0% vs 8.2%, $P < 0.001$) [72]. Finally, non-controlled studies have also been performed to evaluate the effect of combination therapy. A study by Kodama et al. demonstrated a significant improvement in malondialdehyde and 8-OHdG, a marker of DNA oxidation, after prescribing a daily regi-

men of vitamin C, vitamin E, and glutathione [73]. When administered in sufficient quantities, vitamins C and E appear to effectively attenuate seminal oxidative stress and improve markers of DNA damage [74].

Additional antioxidants have been assessed. A recent randomized placebo-controlled trial by Nadjarzadeh and colleagues evaluated the effect of daily administration of coenzyme Q_{10}, a compound that faciliates electron transport in the mitochondrial membrane, mediating cellular synthesis of ATP [75]. These investigators observed improvements in seminal concentrations of malondialdehyde, demonstrating that coenzyme Q_{10} therapy allayed some oxidative stress burden on the male reproductive tract [76]. Others have examined the impact of glutathione supplementation on markers of oxidative stress, given the importance of this compound in neutralizing reactive chemical species in the epididymis [77, 78]. Though few studies have examined the effect of glutathione therapy on markers of oxidative stress, a small non-controlled trial by Lenzi et al. demonstrated improvement in markers of lipid peroxidation [79]. L-carnitine has also been a compound of interest in monotherapy studies. Carnitine is produced by the liver and is essential for transporting fatty acids into the mitochondrial matrix from the cytosol via the carnitine shuttle, a critical process for fatty acid metabolism [80]. Lenzi and colleagues assessed the impact of L-carnitine supplementation on semen parameters following daily administration for 2 months [81]. The therapy failed to improve markers of lipid peroxidation or sperm function tests, raising doubts about its benefit to male fertility. Vicari et al. evaluated the effect of L-carnitine supplementation with administration of non-steroidal anti-inflammatory drugs (NSAIDs) in a population of men with confirmed oxidative stress [82]. The group receiving L-carnitine alone improved the least with regard to reactive oxygen species production and semen parameters. However, the group that received NSAIDs for 2 months followed by L-carnitine administration for 2 months fared best. It is unclear what role L-carnitine may play in relieving the burden of oxidative stress and DNA damage in the male reproductive tract, but it appears combination therapy yields the most promising results.

Though antioxidant therapy has been evaluated as a treatment for oxidative stress, few studies have assessed markers of oxidative stress and sperm DNA fragmentation as outcome variables in the setting of clinical varicocele. However, numerous studies have demonstrated that antioxidant therapy improves semen parameters and may increase the pregnancy rate in couples where the male partner is affected by varicocele. Cavallini and colleagues reached this conclusion in a prospective control study that investigated the effects of cinnoxicam and L-acetyl-carnitine in 325 patients with idiopathic infertility, subclinical varicocele, or clinical varicocele [83]. Patients were organized into three groups: the first intervention arm was treated with carnitine, L-acetyl-carnitine, and cinnoxicam; the second intervention arm was treated with carnitine and L-acetyl carnitine without cinnoxicam; the control arm received glycerine and starch. Patients assigned to the intervention arms experienced improvements in semen parameters and pregnancy rates that were significantly higher than that of the controls, with the cinnoxicam treatment group benefitting most (38.0% vs. 21.8% vs. 1.7%, $P < 0.01$). However, the effects on semen parameters were not sustained after therapy was discontinued, indicating a temporary therapeutic effect. Other studies assessing antioxidant therapy in varicocelized men have similarly demonstrated improvements in semen parameters and pregnancy rates [84].

There is ample evidence that antioxidant therapy improves seminal markers of oxidative stress and oxidative DNA damage. However, the medical community remains skeptical about the efficacy of antioxidant therapy due to the dearth of high-quality, randomized controlled trials demonstrating improvements in live birth rates. Because of this uncertainty, it would be unethical to offer varicocelized men antioxidant therapy in place of corrective surgery, which has more substantial evidence supporting its efficacy in treating infertility in the setting of clinical varicocele. Interestingly, antioxidant therapy

may confer the greatest benefit as an adjuvant to varicocele repair [85, 86]. This is an active area of investigation, with current research focusing on whether adjuvant antioxidant therapy improves markers of oxidative stress, semen parameters, and natural pregnancy rates in the postoperative period.

Conclusion

Dilatation and convolution of the pampiniform plexus results in venous stasis that induces heat stress and hypoxia in the male reproductive tract. As a consequence of the resulting metabolic dysfunction, production of reactive oxygen species and reactive nitrogen species increases, damaging testicular tissue and maturing sperm. Accordingly, men with varicocele have increased markers of oxidative stress in their semen as well as higher levels of sperm DNA fragmentation compared to healthy controls. Surgical correction of varicocele improves both of these measures, with some studies demonstrating sustained normalization of these parameters postoperatively. A wide variety of antioxidant monotherapies and combination therapies that have been studied, most which improved seminal markers of oxidative stress and DNA damage. More research investigating the impact of oral antioxidants on male fertility, especially in the form of randomized controlled trials, is required to reach a more definitive conclusion on the efficacy and clinical utility of medical therapy in the treatment of varicocele.

Review Criteria

We extensively searched Google Scholar, PubMed, Medline, Clinical Key, and Science Direct for articles focusing on varicocele-associated oxidative stress, production of reactive chemical species, modalities of varicocele repair, outcomes of varicocelectomy, and administration of antioxidant therapy as treatment for varicocele. We began our literature search in January 2019 and completed it by February 2019. The following key words were utilized in our search: "varicocele", "clinical varicocele", "varicocelectomy", "varicocele repair", "antioxidant", "antioxidant therapy", "oxidative stress", "sperm DNA fragmentation", "DNA damage", "DNA oxidation", "reactive oxygen species", "reactive nitrogen species", "heat stress", and "hypoxia".

Multiple Choice Questions and Answers

1. Oxidative stress in the setting of varicocele can be exacerbated by:
   (a) Increased cellular concentrations of glutathione
   (b) Increased total antioxidant capacity
   (c) Reduced production of reactive nitrogen species
   (d) **Disruption of the electron transport chain**
2. Varicocele-induced heat stress:
   (a) Decreases seminal markers of oxidative stress
   (b) Results from increased arterial blood flow through the testis
   (c) **Increases the percentage of sperm positive for DNA fragmentation**
   (d) Most commonly results from right-sided varicocele
3. Fertility is most definitively assessed using which of the following outcome variables?
   (a) DNA fragmentation index
   (b) Markers of lipid peroxidation
   (c) **Live-birth rate**
   (d) Total antioxidant capacity
4. Varicocele repair with surgery or percutaneous embolization has been shown to:
   (a) Improve measures of sperm DNA fragmentation
   (b) Improve markers of lipid peroxidation
   (c) Improve markers of oxidative DNA damage
   (d) **All of the above**

5. Which of the following is NOT an antioxidant used to treat oxidative stress?
   (a) Vitamin C
   (b) **Clomiphene**
   (c) L-carnitine
   (d) Coenzyme Q_{10}

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Effect of Varicocele Treatment on Natural Pregnancy Outcomes

23

Siddharth Yadav and Rajeev Kumar

Key Points

- As compared to artificial reproductive techniques, natural pregnancy has lower risk of gestational hypertension, diabetes, placenta previa and placental abruption.
- Varicocelectomy is beneficial in men with clinical varicoceles and impaired semen analysis with normal female partner evaluation.
- There is no benefit of varicocelectomy on pregnancy rates in men with subclinical varicoceles or those with normospermia.
- Age, BMI, grade of varicocele, pre- and post-operative sperm counts have been shown to affect outcomes in men undergoing varicocelectomy.
- Microsurgical subinguinal varicocelectomy has the highest post-surgery natural pregnancy rates.

Couples resort to infertility evaluation if they are unable to conceive naturally and no matter how difficult or simple the treatment is, natural conception is always favoured by the patients and the caregivers alike [1]. With advancements in in-vitro fertilisation (IVF) techniques and the advent of intra-cytoplasmic sperm injection (ICSI), an overall delivery rate of 18.4–25.2% has been reported amongst various causes of infertility [2, 3]. However, the pregnancies achieved by IVF are at a higher risk of obstetric and perinatal complications as compared to natural pregnancies. Compared with a natural pregnancy, post-IVF pregnant women are at a twofold higher risk of hypertension, twofold higher risk of diabetes, three- to sixfold higher risk of placenta previa and a twofold higher risk of placental abruption [1, 4–6]. The chances of inducing the labour or undergoing a Caesarean section are also two times higher amongst pregnancies following IVF. Post-IVF pregnant females also have a worse perinatal outcome. There is a twofold higher risk of stillbirth or neonatal death, one- to twofold higher risk of preterm delivery, twofold higher risk of low birth weight (<2500 gm) and a two- to threefold higher risk of very low birth weight (<1500 gm), one- to twofold higher risk of small for gestational age and one- to twofold higher risk for NICU admission [1, 4–6].

The increased chance of multifetal pregnancy post IVF further worsens these obstetric and perinatal outcomes [7]. Mothers of IVF multiple

S. Yadav
Department of Urology and Renal Transplant, Vardhaman Mahavir Medical College & Safdarjung Hospital, New Delhi, India

R. Kumar (✉)
Department of Urology, All India Institute of Medical Sciences, New Delhi, India
e-mail: rajeev.urology@aiims.edu

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pregnancies are also more likely to have a dysfunctional parent–child interaction and the perception of child difficulty [8]. In terms of cost, varicocelectomy followed by natural pregnancy is more cost-effective as compared to IVF [9]. Amongst patients with a history of infertility, who go on to have an unassisted natural pregnancy, the obstetrical and perinatal outcomes are poorer than couples who have never had a history of infertility. There is a twofold increase in pre-eclampsia, placental abruption, Caesarean section and vacuum extraction rates and a threefold increased risk in perinatal mortality rates [10, 11].

Around 1% of all the fertile couples suffer from recurrent miscarriages, the cause of which cannot be established in around half of the cases despite extensive evaluation [12]. One of the presumed causes of the higher miscarriage rate is the greater likelihood of chromosomal abnormalities amongst men with oligo or azoospermia. Varicoceles are associated with elevated reactive oxygen species levels and reduced seminal antioxidant capacity which can result in higher oxidative damage to sperm DNA [13, 14]. Excessive DNA damage has been associated with poor fertility indices such as embryo cleavage rates, implantation rates, pregnancy rates and live birth rates [15]. Surgical varicocelectomy has been shown to reduce ROS production, increases antioxidant levels and reduces abortion rates [16]. In a study of 136 patients with history of recurrent abortions, normal husband semen parameters and clinical varicoceles, varicocelectomy improved natural pregnancy rates and reduced abortion rates [17].

Effects of Varicocelectomy on Natural Pregnancy Rates: What is the Literature?

Varicocelectomy for infertility has always been a debated topic and one of the reasons is the lack of robust end-points for evaluating success. While improvements in semen parameters remain the most common surrogate marker for success, the primary aim of the treatment is a pregnancy leading to a live birth, not the seminal improvements. Coupled with a background pregnancy rate of 19.9% over 1 year in couples with infertility who do not undergo any treatment, any intervention for varicoceles should demonstrate outcomes higher than this in order to be called successful [18]. Despite the documented improvement in semen parameters, the benefit of varicocelectomy on natural pregnancy rate is controversial [19].

A large number of factors related to both male and female partners affect natural pregnancy and thus may confound the evaluation of the benefits of varicocelectomy. Disease-specific parameters such as subclinical vs clinical varicoceles, normal vs abnormal semen parameters, grade of varicoceles, unilateral vs bilateral may also have an impact on the final outcomes.

Tulloch in 1952 first documented how the surgical repair of varicocele in a man with azoospermia resulted in a natural pregnancy, thus providing the evidence of a link between varicocele and infertility [20]. Madgar et al. supported this association in one of the first trials reporting the effect of varicocelectomy on the natural pregnancy rates [21]. They enrolled 45 couples with infertile men with abnormal semen parameters with varicocele as the only demonstrable cause of infertility and divided them into 2 groups. Group A (20 couples) were kept under observation for 1 year followed by high ligation of varicocele in the male partner after the first year and Group B (25 couples) in which the male partner underwent upfront varicocele treatment. The difference in pregnancy rates within the first year in both the groups was highly significant (10% in Group A vs 60% in Group B). Further, within Group A, the pregnancy rate increased from 10% before ligation to 44% after ligation. The authors also noted a significant improvement in semen parameters regardless of the pregnancy and concluded that varicoceles are clearly associated with infertility and their correction by ligation improves semen parameters and fertility rates. Furthermore, the highest pregnancy rates in both groups occurred during the first year post surgery.

In 1996, Yamamoto et al., in their randomised controlled study, evaluated the role of varicocele ligation on fertility and semen parameters in 85 infertile men with oligozoospermia with subclinical varicoceles diagnosed by scrotal thermography [22]. They noted significantly higher

levels of sperm density and total motile sperm count at 1 year post intervention. However, the pregnancy rate (6.7% vs 10%; $p = 0.75$) and other semen parameters did not improve significantly and hence they concluded that subclinical varicocelectomy has no beneficial effects on natural pregnancy rates.

In 1998, Nieschlag et al. recruited 125 infertile couples with clinically palpable varicoceles and subnormal semen parameters and randomised them into 2 groups (group 1 – 47 patients – 23 ligations and 24 embolisations and group 2 – 48 patients – observation) [23]. At 1 year follow-up, there was an improvement in sperm concentration in group 1; however, there was no difference in the pregnancy rates (25.5% in intervention vs 27.1% in observation arm). Based on these results the authors challenged the association between infertility and varicocele and urged for the need of properly designed controlled trials.

The Cochrane Controversies

To answer this question, the Cochrane Collaboration performed a meta-analysis in 2001 of the then available 5 randomised trials (including the above 3) [24]. Of the other 2 RCTs, one included men with subclinical varicocele and the definition of subfertility was not stated, whereas the other included men with clinical varicocele with subfertility; however, normozoospermia was not an exclusion criterion and only 26% men actually had sperm counts <20million/ml [25, 26]. Of the evaluated studies, except for one RCT which showed a huge and significant benefit (Madgar et al), none of the other four studies individually or in combination showed a significant effect of varicocele treatment on the pregnancy rates over no-treatment and the combined relative risk (RR) (random effects method) favouring treatment over no treatment (or counselling) of the five studies combined was 1.06. Based on these results the authors concluded that surgical or radiological treatment of varicocele in men from couples with otherwise unexplained subfertility cannot be recommended. However, this analysis was criticised for including men with subclinical varicoceles and normal parameters and thus the true benefits of varicocelectomy may not have been achieved [27].

In the subsequent years, 2 more RCTs echoed similar non-significant results. Unal et al. randomised 42 patients with subclinical varicoceles and infertility to varicocelectomy or clomiphine citrate [28]. At a median follow-up of 15 months, only 3 pregnancies were noted in the whole cohort and the authors could not elucidate any significant benefit of varicocelectomy compared to medical treatment on pregnancy rates (12.5% vs 6.7% $p = 0.5$). Krause et al. randomised a cohort of 67 patients with clinical varicoceles and at least one abnormal semen parameter to either sclerotherapy or observation [29]. At a follow-up of 12 months, the difference in clinical pregnancy rates between the intervention (30%) and the observation (16.2%) arms was insignificant ($p = 0.189$). However, in this study, some patients who were initially randomised to observation arm were actually treated and an analysis based on intention to treat also did not yield a significant difference in pregnancy rates. Further, their study was marred by poor accrual. Of the 460 patients that were planned, only 67 could be recruited and inadequate power of the study was stated as one of the reasons of insignificant results by the authors.

In the light of these studies, Cochrane Collaboration again analysed the available literature in 2004 of the then available 8 studies and recommended against the benefit of varicocele treatment over expectant management in improving the pregnancy rates [30]. Despite the ongoing research, no new RCTs were published till 2009 and thus the 2009 Cochrane Collaboration meta-analysis ended up re-evaluating the same 8 studies with the same results [31, 32]. However, both these analyses had also included men with subclinical varicoceles and normal semen parameters like its predecessor.

To overcome these limitations, Ficarra et al. in 2006 reanalysed the Cochrane Collaboration 2004 review and excluded the patients with subclinical varicoceles or normal semen parameters [33]. Only 3 of the 8 studies included patients with abnormal semen parameters and clinical varicoceles [21, 23, 29]. The authors noted

a significant improvement in pregnancy rates in the treatment (36.4%) vs the control (20%) arm ($p = 0.009$). They concluded that the RCTs included in the last Cochrane review were heterogenous and methodologically poor and pooling of these studies could not have resulted in a good-quality meta-analysis. The result demonstrated the role of varicocelectomy in men with abnormal semen parameters and clinical varicoceles.

In 2007, Marmar et al. analysed 2 RCTs and 3 observational studies evaluating the role of varicocelectomy in men with abnormal semen parameters and palpable varicoceles [34]. Because of the paucity of randomised trial data, the authors included observational studies in their analysis. In their meta-analysis they reported a pregnancy rate of 33% in the treatment arm vs 15.5% in the control arm; the difference was clinically significant ($p = 0.007$) and the number needed to treat was 5.7. To safeguard against methodological bias in the observational studies, the authors used the same scoring system which is used for quality assessment of randomised trials while deciding for their inclusion in the meta-analysis.

Microsurgical varicocelectomy is considered the gold standard technique as the magnification provided by microsurgical techniques or by use of loupes affords better visualisation of the vasculature and improves the outcomes [35]. Two RCTs specifically assessed the effects of microsurgical or loupe-assisted varicocelectomy versus observation on the natural pregnancy rates. Abdel-Meguid et al. enrolled 145 participants 20–39 yrs. of age with palpable varicoceles, at least one abnormal semen parameter and infertility [36]. Seventy-three patients underwent subinguinal microsurgical varicocelectomy and 72 underwent observation. At a follow-up of 1 year, natural pregnancy was achieved in 32.9% in the treatment arm vs 13.9% in the control arm ($p = 0.010$), with an odds ratio of 3.04. The authors provided level 1b evidence for the superiority of varicocelectomy over observation in infertile men with palpable varicoceles and impaired semen quality. Another study by Mansour Ghanaie et al. randomised 136 couples with history of recurrent abortions, clinical varicoceles but normal semen parameters into loupe-assisted inguinal varicocelectomy vs observation and followed them for a period of 12 months [17]. The overall pregnancy rate in the treatment arm was 44.1% as compared to 9.1% in the observation arm at 12 months of follow up ($p = 0.003$). Further, of the women who conceived, the miscarriage rate was 13.3% in the treatment group vs 69.2% in the observation group ($p = 0.001$). The authors concluded that varicocelectomy improves pregnancy rates and decreases the miscarriage rate significantly.

In the light of these new RCTs, Bazeem et al. re-analysed the available literature reporting on the pregnancy outcomes after varicocelectomy [37]. They evaluated 4 RCTs [21, 23, 29, 36] totalling 380 couples with clinical varicocele and oligozoospermia. The fixed-effect model combined odds ratio was 2.10 ($p = 0.002$) suggesting that varicocelectomy was superior to observation. However, the Q statistics p value was 0.024, indicating non-homogeneity amongst the evaluated studies. Indeed, the study by Madgar et al. had strikingly high pregnancy rate in the treatment arm as compared to the other studies; the study by Krause et al. suffered from poor accrual, high dropout and considerable loss to follow-up; the study by Abel Meguid et al. had surprisingly low number of pregnancies in the control arm and that by Nieschlag et al. had high dropout rates. Thus the authors used random effect model and the combined odds ratio was 2.23 ($p = 0.09$), indicating that there was a beneficial effect of varicocelectomy, but it did not reach statistical significance. The authors, thus, finally concluded that there is an insufficient evidence to demonstrate the beneficial effect of varicocelectomy on pregnancy rates. Following this, the Cochrane Collaboration again reviewed the available data and this time 10 RCTs were included, 3 of them exclusively evaluated men with subclinical varicoceles and 2 trials included some men with normal semen parameters [38]. From the 894 men that were included, the authors found that the random effect odds ratio for the outcome of pregnancy was 1.47 ($p = 0.03$) favouring the intervention and the number needed to treat was 17; suggesting limited benefits of varicocelectomy. However, in a pre-planned sub-group analysis of trials that included only men with clinical varicoceles and abnormal semen analysis (5 RCTs), the odds ratio was 2.39 ($p = 0.03$) favouring varicocelectomy and the number needed to treat was 7; a

much better benefit ratio than when evaluating the entire population. The authors concluded that surgical or radiological treatment of men with clinical varicocele and abnormal semen analysis may be of clinical benefit, but the evidence is not conclusive. They also stated that the value of surgical or radiological treatment in subfertile men with subclinical varicoceles and normal semen analysis is disputable.

The most recent meta-analysis has focused on the surgical treatment of varicoceles (excluded endovascular treatment) and included 7 RCTs with 610 infertile men including those with subclinical varicoceles and normal semen parameters [39]. The random effects model showed an odds ratio of 1.90 favouring varicocelectomy however, this was not statistically significant ($p = 0.162$). On sub-analysis of 3 RCTs that included only patients with clinical varicocele and impaired semen quality, the fixed-effect pooled odds ratio was 4.15 favouring varicocelectomy which was statistically significant ($p < 0.001$). The authors concluded that surgical varicocelectomy can play a significant role in improving pregnancy rates when performed in men with clinical varicoceles and impaired semen analysis (RCTs and meta-analyses have been summarised in Tables 23.1 and 23.2 respectively).

To summarise, the current evidence suggests that there is no benefit of varicocelectomy on pregnancy rates in men with varicoceles with normal semen parameters or those with subclini-

Table 23.1 Summary of randomised trials on the effect of varicocelectomy on natural pregnancy rates

Author and year of publication	Population studied	Intervention and control	Natural pregnancy outcomes	Remarks
Madgar et al. (1995) [21]	45 couples with infertility, clinical varicoceles and abnormal semen parameters	25 underwent immediate high ligation (Grp A); 20 were observed for 1 year followed by high ligation (Grp B)	Grp A: 60% vs Grp B:10% ($p = 0.001$); Within Grp B – 10% before and 44% after ligation	Highest pregnancy rates occur within the first year after ligation
Yamamoto et al. (1996) [22]	85 couples with infertility, oligospermia and subclinical varicoceles diagnosed on scrotal thermography	45 underwent high ligation (Grp A); 47 were observed (Grp B)	Grp A: 6.7% vs Grp B: 10% ($p = 0.758$)	No benefit of varicocelectomy in men with subclinical varicoceles
Nieschlag et al. (1998) [23]	125 couples with clinical varicoceles and subnormal semen parameters	47 interventions (23 ligations, 24 embolisations) (Grp A), 48 observation (Grp B)	Grp A: 25.5% vs Grp B: 27.1% (p = NS)	No benefit of varicocelectomy
Unal et al. (2001) [28]	42 patients with left subclinical varicoceles	21 varicocelectomy (Grp A), 21 clomiphene citrate (Grp B)	Grp A: 12.5% vs Grp B: 6.7% ($p = 0.5$)	No difference in medical and surgical management on pregnancy rates
Krause et al. (2002) [29]	67 patients (2 dropouts) with clinical varicocele and at least one abnormal semen parameter	32 embolisation (Grp A), 33 observations (Grp B)	Grp A:30% vs Grp B: 16.2% ($p = 0.189$)	Poor accural, high dropout rates
Abel-Meguid et al. (2011) [36]	145 men with palpable varicocele and at least one abnormal semen parameter	73 microsurgical varicocelectomy (Grp A), 72 observation (Grp B)	Grp A: 32.9% vs Grp B: 13.9% ($p = 0.10$)	Microsurgical varicocelectomy improves pregnancy rates
Mansour Ghanaie et al. (2012) [17]	136 couples with recurrent abortions and clinical varicoceles with normal semen parameters	68 loupe-assisted varicocelectomy (Grp A); 68 observation (Grp B)	Grp A: 44.1% vs Grp B: 19% ($p = 0.003$)	Significantly lower miscarriage rates in those who underwent varicocelectomy (13.3% vs 69.2%)

Table 23.2 Summary of meta-analyses evaluation the effect of varicocelectomy on natural pregnancy rates

Meta-analysis and year of publication	Population studied	Intervention and control	Natural pregnancy outcomes (treated vs untreated)	Remarks
Evers et al. (2001) [24]	5 RCTS 430 couples with varicoceles	Surgical ligation or embolisation (226 pts) vs no treatment (204 pts)	25% vs 22.7% OR - 1.15	No benefit of varicocelectomy Patients with subclinical varicoceles and normospermia were also included
Evers et al. (2004, 2009) [30, 31, 32]	8 RCTS 607 couples with varicoceles	Surgical ligation or embolisation (314 pts) vs no treatment (293 pts)	21% vs 19.1% OR - 1.10 ($p = 0.0$)	No benefit of varicocelectomy Patients with subclinical varicoceles and normospermia were also included
Ficarra et al. (2006) [33]	3 RCTS 237 couples with palpable varicoceles and abnormal semen analysis	Surgical ligation or embolisation (120 pts) vs no treatment (117 pts)	36.4% vs 20% $p = 0.009$	Varicocelectomy beneficial in patients with clinical varicocele and abnormal semen parameters
Marmar et al. (2007) [34]	2 RCT and 3 observational studies 570 couples with palpable varicoceles and abnormal semen analysis	Surgical ligation (396 pts) vs no treatment (174) pts	33.0% vs 15.5% OR - 2.87 $p = 0.007$	Varicocelectomy beneficial in patients with clinical varicocele and abnormal semen parameters
Bazeem et al. (2011) [37]	4 RCTs 380 couples with palpable varicoceles and oligospermia	Surgical ligation or embolisation (192 pts) vs no treatment (188 pts)	32.2% vs 18% OR - 2.23 $p = 0.091$)	Varicocelectomy is moderately superior but statistically insignificant
Kroese et al. (2012) [38]	10 RCTs 894 couples with varicoceles	Surgical ligation or embolisation (449 pts) vs no treatment (445 pts)	23.1% vs 17.3% OR - 1.47 $p = 0.03$	Pre-planned subgroup analysis of studies with palpable varicoceles and abnormal semen analysis showed an OR = 2.39 ($p < 0.0001$)
Kim et al. (2013) [39]	7 RCTs 610 couples with varicoceles	Surgical ligation (311 pts) vs no treatment (299) pts	21.8% vs 11.0% OR - 1.90 $p = 0.1621$	Subgroup analysis of 3 RCTs that included patients with clinical varicocele and and abnormal semen parameters showed an OR of 4.14 ($p < 0.001$) favouring varicocelectomy

cal varicoceles. However, in men with clinical varicoceles and impaired semen analysis, varicocelectomy may be beneficial but the quality of supporting evidence is limited.

Factors Predicting Outcomes

Several prognostic factors have been identified that may help predict favourable outcomes after varicocelectomy, both in terms of improvement in the semen parameters and the fertility rates [40]. The age at surgery, grade of varicocele, pre-operative semen parameters, pre-operative serum FSH levels, testicular volume and other factors have been shown to affect the post-operative improvements in semen parameters [40]. But like the effect of varicocelectomy on pregnancy rates, the factors that influence post-operative pregnancy rates are also not well defined.

Amongst the infertile males with varicoceles undergoing surgical intervention, younger age predicts better post-surgical improvement in semen parameters whereas a high body mass index and a decrease in serum FSH negatively affects the natural pregnancy rates [41–43]. Duration of

infertility also negatively correlates with natural pregnancy rates and those with the shortest duration (0–3 years) of infertility have the highest natural pregnancy rates [44]. A positive correlation between the grade of varicoceles and the natural pregnancy rates has not been established. Men with grade 3 varicoceles have a greater degree of improvement in semen parameters as compared to men with grade 1 or grade 2 varicoceles; however, post-intervention pregnancy rates are not statistically different [45]. Men with bilateral varicoceles tend to have greater improvements in natural pregnancy rates post varicocelectomy as compared to those with unilateral varicoceles [46, 47]. However, in men with unilateral clinical and contralateral subclinical varicocele, no significant differences were found in the post-operative natural pregnancy rates between men who underwent bilateral varicocelectomy as compared to unilateral varicocelectomy [48]. Thus the current evidence suggests varicocele repair only in patients with clinical varicoceles. Baseline semen parameters have also been shown to affect post-operative pregnancy rates. A pre-operative sperm concentration greater than or equal to 5 million/ml and a post-operative motile sperm count of >20million correlate with a higher post-operative natural pregnancy rate [49, 50]. Female partner's age has also been suggested as one of the important predictors of pregnancy outcomes as a younger female partner age correlated with higher pregnancy rates in one of the earlier series [23]. However, subsequent studies found similar natural pregnancy and overall pregnancy rates amongst infertile couples with a female partner age of >35 years as compared to <35 years [51]. Varicocelectomy may be a more cost-effective option, and thus it can be considered an acceptable alternative even in couples with advanced female age.

Effects of Surgical Approach

Various surgical procedures have been described for varicocele ranging from the open, laparoscopic, microsurgical techniques to percutaneous embolisation. Each procedure has its own success and complication rates, and has been shown to produce various degrees of improvement in semen parameters and natural pregnancy rates [52]. Many studies have compared the surgical outcomes of these various approaches; however, only a few randomised trials have included natural pregnancy in the outcome assessment. Al-Said et al. in a randomised study compared open, laparoscopic and microsurgical approaches of varicocelectomy in 298 infertile males with clinical varicoceles: 92 underwent open inguinal varicocelectomy, 94 had laparoscopic and 112 had microsurgical repair of varicoceles [53]. At a mean follow-up of 21 months, all patients had a significant improvement in semen parameters as compared to the baseline. The magnitude of improvement was greatest amongst those who underwent microsurgical repair; however, the natural pregnancy rates were comparable, 31%, 33% and 38% in the open, laparoscopic and microsurgical groups respectively. In a similar study, Al-Kandari et al. randomised 120 patients to one of the three varicocelectomy techniques, open, laparoscopic or microsurgical [54]. At a mean follow-up of 18 months, the improvement in the semen parameters and the natural pregnancy rates (open 28%, laparoscopic 30% and microsurgical 40%) were comparable between the 3 groups. Nasr et al. performed a prospective trial comparing 49 men who underwent microsurgical varicocelectomy and 27 men who underwent embolisation for infertility and clinical varicocele [55]. At a mean follow-up of 4 years, there was no difference between the two procedures in respect with sperm quality, pregnancy rates and overall satisfaction rates.

A recent meta-analysis included 56 studies and stratified the surgical outcomes according to the procedure type [52]. Sperm concentration improved after all the procedure types; the highest improvement was seen after laparoscopic transabdominal varicocelectomy (by 19.8×10^6/ml). The authors also evaluated natural pregnancy rates which ranged from 26% to 41% amongst the various procedures. The highest natural pregnancy rate was seen after microsurgical subinguinal varicocelectomy (41%). The other open approaches, inguinal and retroperitoneal, showed a natural pregnancy rate of 26% and 37% respectively. The

natural pregnancy rate after laparoscopic transabdominal approach was 26% whereas it was 36% after endovascular embolisation. Although a direct comparison between the procedures is not possible because of the heterogenous study population, the authors still concluded that the microsurgical varicocelectomy affords the highest natural pregnancy rates. Also, the data available on microsurgical varicocelectomy are much more robust and consistently report good fertility outcomes, whereas, endovascular embolisation is mostly reported from the few centres with relatively small patient number and generalisability of these outcomes may be difficult.

Conclusions

Natural pregnancy is the desired goal behind every varicocele treatment and varicocele repair is considered a better and economically cheaper option compared to ART. Varicocelectomy, whether surgical or endovascular, has been shown to improve natural pregnancy rates in men with clinical varicoceles with abnormal semen parameters but benefit in settings of normal semen parameters or subclinical varicoceles has not been well demonstrated. Young, non-obese men with bilateral grade 3 varicoceles without testicular atrophy and short duration of infertility with young age of female partner are thought to have maximal benefit. Microsurgical subinguinal varicocelectomy has the highest post-surgery natural pregnancy rates and is the current recommendation; however, direct comparison studies are few and lacking.

Review Criteria

This review is based on a search of studies examining the relationship between varicocelectomy and natural pregnancy using PubMed. The search was based on the following key words: “varicocele”, “varicocelectomy”, “infertility”, “pregnancy rates”, “natural pregnancy”. Articles published in language other than English were not evaluated and all articles published to date were evaluated. Data that were solely published in conference or meeting proceedings, websites or books were not included.

Multiple Choice Questions and Answers

1. Varicocelectomy improves natural pregnancy rates in men with
   (a) Subclinical varicoceles with azoospermia
   (b) Normal semen parameters with grade III varicocele
   (c) **Palpable varicoceles with oligospermia**
   (d) Oligospermia with varicocele detected on scrotal thermography
2. Highest natural pregnancy rates after varicocelectomy is seen after
   (a) **Microsurgical varicocelectomy**
   (b) Open retroperitoneal varicocelectomy
   (c) Anterograde embolisation
   (d) Laparoscopic varicocelectomy
3. Which of the factors has not been shown to affect post varicocelectomy natural pregnancy rates?
   (a) Pre-operative sperm counts
   (b) Post-operative sperm counts
   (c) Testicular volume
   (d) **Grade of varicocele**
4. Varicocelectomy for clinically palpable varicoceles may
   (a) Improve semen parameters
   (b) Improve natural pregnancy rates
   (c) Reduce abortion rates
   (d) **All of the above**
5. Treatment of varicoceles is recommended in an infertile couple if
   (a) Associated abnormal semen parameters
   (b) Varicocele is clinically palpable
   (c) The female partner evaluation is normal
   (d) **All of the above**

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24 Effect of Varicocele Treatment on Assisted Reproductive Technology (ART) Pregnancy Outcomes

Joseph Scott Gabrielsen, Nannan Thirumavalavan, and Alexander W. Pastuszak

Key Points

- Repair of clinical varicoceles is associated with improvements in clinical pregnancy and live birth rates in couples undergoing intrauterine insemination, in vitro fertilization, and IVF-ICSI.
- Repair of clinical varicoceles can significantly decrease the degree of assistance needed to achieve pregnancy.
- The costs (both financial and time) associated with varicocele repair remain understudied.
- These data should be considered and discussed with couples prior to ART when a clinical varicocele is detected in the male partner.

J. S. Gabrielsen (✉)
Division of Male Reproductive Medicine and Surgery, Scott Department of Urology, Baylor College of Medicine, Houston, TX, USA

N. Thirumavalavan
Urology Institute, University Hospital / Case Western Reserve University, Cleveland, OH, USA

A. W. Pastuszak
Division of Urology, Department of Surgery, University of Utah School of Medicine, Salt Lake City, UT, USA

Introduction

Varicoceles are more common among men with infertility than in the general population. In the era of in vitro fertilization-intracytoplasmic sperm injection (IVF-ICSI), where few sperm are needed to be able to fertilize an egg, whether infertile men with varicocele should undergo varicocelectomy to improve semen parameters prior to attempting conception using assisted reproductive technologies (ART) may be questioned. Germane to this issue, however, are two questions: (1) does varicocelectomy improve ART outcomes and (2) can varicocelectomy decrease the need for ART?

In this chapter, we examine the literature regarding the impact of varicocele repair on ART outcomes. Specifically, we focus on the outcomes of repairing clinically detectable varicoceles, as current guidelines recommend against the repair of subclinical varicoceles and a recent meta-analysis found little benefit in doing so [1, 2].

The primary outcomes of interest are changes in fertilization, pregnancy and live birth rates, and changes in the need for ART as a result of varicocele repair. As the effects of varicocelectomy on semen parameters and sperm quality have been discussed extensively in previous chapters, we will only address changes in total motile sperm count (TMSC), as this outcome is meaningful when determining whether couples continue to attempt natural conception or proceed to intrauterine insemination (IUI) or IVF/IVF-ICSI.

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Varicocelectomy Prior to Intrauterine Insemination

Limited literature exists examining the effect of varicocelectomy on IUI outcomes. While many available studies investigating the effects of varicocele repair in the setting of ART include some patients that undergo IUI, their primary focus has been on IVF/IVF-ICSI outcomes. These will be addressed in subsequent sections. Two studies, however, specifically investigated the effects of varicocelectomy on IUI outcomes.

Marmar et al. published a study of 66 men with history of varicocele who proceeded to IUI; 52 of the men underwent varicocelectomy and 14 did not [3]. In the varicocelectomy group, there were four pregnancies (4/52, 7.7%) compared to two (2/14, 14.3%) within the control group; however, this difference was not statistically significant (p value not reported). It should be noted that the low pregnancy rates observed likely resulted from the women not undergoing ovarian stimulation prior to IUI. Additionally, two of the pregnancies were initiated by men with normal semen parameters and it is unknown to which group those men belonged.

In contrast, Daitch and colleagues retrospectively evaluated 58 couples with men with varicocele who had undergone IUI; 34 of these men underwent varicocelectomy while 24 elected conservative management [4]. While the odds ratio (OR) of pregnancy per cycle (OR 1.87, 95% CI 0.53–8.28) and per couple (OR 2.12, 95% CI 0.55–10.02) favored varicocele repair, the difference was not significant. When other factors known to affect fertility were included in a multivariate analysis, however, men who underwent varicocelectomy had significantly higher odds of pregnancy (OR 4.4, 95% CI 1.1–17.8) and live birth (OR 23.6, 95% CO: 2.3–237.5), strongly favoring repair ($p = 0.04$ and 0.007, respectively).

Varicocelectomy Prior to IVF/IVF-ICSI

Over the past 30 years, multiple studies have examined the effects of varicocele repair on IVF/IVF-ICSI outcomes. The first report of the effect of varicocelectomy on IVF outcomes was published by Ashkenazi and colleagues, who reported on 22 couples with a prior history of IVF failures [5]. All men subsequently underwent varicocele repair. The authors report that after repair, 20% of the couples with a female factor ($n = 12$) and 40% of those without a female factor ($n = 10$) were able to achieve pregnancy through additional IVF cycles. While this study lacked a non-varicocelectomy control group, it provided evidence that there may be a benefit for varicocelectomy prior to IVF.

Likewise, in 1994, Yamamoto et al. reported on 13 couples who had failed IVF prior to the men undergoing varicocele repair [6]. On subsequent IVF cycles, the oocyte fertilization rate increased from 9.8% preoperatively to 41% postoperatively. This increased fertilization rate led to a greater number of embryos transferred and, ultimately, successful pregnancies. This study, however, also lacked a non-varicocelectomy control.

When IVF-ICSI is used, a single sperm is injected directly into an oocyte. Thus, it may not be surprising that Shiraishi and colleagues found no significant differences in fertilization rates in 21 men who underwent varicocelectomy compared to 53 who had varicoceles but did not undergo surgery [7]. The authors did observe, however, significant improvements in clinical pregnancy and live birth rates in the varicocelectomy group (61.9% vs 28.3% and 52.3% versus 24.5%; $p = 0.02$ and 0.04, respectively).

Esteves et al. reported on IVF-ICSI results in 242 men with clinical varicocele [8]. Of note, none of the couples had attempted IUI or IVF/IVF-ICSI previously. In this study, fertilization rates were higher among couples with men who underwent varicocelectomy (78% vs 66%). Clinical pregnancy rates and live birth rates were also significantly higher in couples with men who did than those who did not undergo repair (60% versus 45% and 46.2% versus 31.4%; $p = 0.04$ and 0.03, respectively). Looking at the data another way, the men who underwent varicocele repair were 1.82 times more likely to achieve clinical pregnancy and 1.87 times more likely to have a live birth compared to those who

did not. Reassuringly, there was also a decreased risk of miscarriage (OR 0.43).

Consistent with these prior studies, Gokce et al. reported on a larger cohort of 306 couples who underwent IVF-ICSI [9]. Men who underwent varicocelectomy had significantly higher TMSC compared to those who were conservatively managed. Pregnancy and live birth rates were also significantly higher in couples with men who had undergone varicocele repair (62.5% versus 47.1% and 47.6% versus 29%; $p = 0.001$ and 0.0002, respectively). Thus, couples in which the men underwent varicocelectomy were approximately twice as likely to have a clinical pregnancy and live birth than those who did not.

Not all studies have reported benefits to varicocelectomy. Pasqualotto et al. reported on IVF-ICSI outcomes of 248 couples with men having history of Grade III varicocele who had not attempted ART, some of whom had previously undergone varicocele repair [10]. The authors observed no significant differences in semen parameters between men who had undergone varicocelectomy compared to those who had not, and fertilization rates were higher in the men who had not undergone repair (73.2% versus 64.9%). No significant differences were observed in pregnancy rate, implantation rates or miscarriage rates. There were significant differences in the duration of infertility between the two groups, though, as those who underwent varicocele repair had an average duration of 6.0 years compared to 2.7 years for those who did not. As a result, it is unclear whether a bias due to more severe fertility defects in the repair group was present.

Likewise, Zini et al. retrospectively reviewed data from 610 couples in which the male had a clinically palpable varicocele, of which 363 of these men elected varicocele repair [11]. While varicocele repair was associated with increased TMSC, no significant difference was observed in natural and overall pregnancy rates between men who underwent repair compared to those who did not. In interpreting these results, however, it should be noted that while TMSC improved with repair, the varicocelectomy group had significantly lower TMSC compared to the controls preoperatively, and postoperatively the TMSC were roughly equivalent between the groups. Thus, it appears that varicocelectomy may have helped those with inferior sperm counts improve to the level of those who did not have surgery.

A recent meta-analysis by Kirby et al. that included the Ashkenazi, Esteves, Gokce and Pasqualotto studies found the OR among oligozoospermic men undergoing varicocele repair prior to IVF/IVF-ICSI was 1.695 (95% CI 0.951–3.020) in favor of pregnancy and 1.699 (95% CI 1.020–2.831) in favor of live birth [12]. A systematic review that included the Esteves, Gokce, Pasqualotto and Shiraishi studies reported the OR to be 1.59 (95% CI 1.19–2.12) for pregnancy and 2.17 (95% CI 1.55–3.06) for live birth [13]. The Zini study may not have been included in either of these analyses due to a large number of individuals lost to follow-up.

Varicocelectomy and Reduction of ART Need

While the above data suggest varicocelectomy improves pregnancy rate and live birth rate in couples undergoing ART, several authors have investigated whether varicocelectomy can improve semen parameters sufficiently to reduce the level of ART needed to achieve pregnancy. As many of the above studies retrospectively looked at individuals who underwent IVF-ICSI, men whose semen parameters improved sufficiently to permit IUI or natural conception would not have been included in the cohort. Thus, if pregnancy can be attained without IVF/IVF-ICSI, the benefits to varicocele repair may be even greater than that reported in analyses by Kirby and Esteves [12, 13].

Varicocelectomy and Reduction of ART Need: Non-Obstructive Azoospermia

The impacts of varicocelectomy on return of sperm in the ejaculate among men with non-obstructive azoospermia (NOA) have been discussed previously and the reader is referred to

those chapters for a more detailed discussion. Some studies have shown that varicocelectomy can increase the likelihood of finding sperm on testicular sperm extraction (TESE), allowing otherwise hopelessly infertile men to father their own children [14, 15]. More relevant to this chapter, however, is literature indicating that varicocelectomy can decrease the intensity of ART needed to attain pregnancy in men with NOA.

A meta-analysis by Weedin et al. summarized the literature prior to 2010 and found that 39% of men with NOA could develop sperm in the ejaculate postoperatively and that 6% were able to conceive naturally without ART [16]. Thus, nearly 4 in 10 men with NOA could avoid testicular sperm extraction and 6% could avoid ART entirely. The effects of varicocelectomy can be short-lived, however, as 11 of the 91 men who regained sperm in the ejaculate subsequently relapsed to azoospermia by 6 months postoperatively.

Varicocelectomy and Reduction of ART Need: Oligozoospermia

Çayan et al. reported on the results of 540 infertile men who underwent varicocele repair [17]. All included men had at least a 1-year history of infertility (mean duration 4.1 +/− 3.7 years). Varicocelectomy was associated with improvements in semen parameters in 271 of the men. Remarkably, 36.6% of men were able to naturally conceive during follow-up, with the majority of pregnancies occurring 3–11 months after repair. Thirty-one percent of men in couples that would have only been IVF-ICSI candidates were reclassified as candidates for natural pregnancy or IUI based on sperm counts. For IVF candidates, 53% improved sufficiently to downgrade to IUI or natural pregnancy. For IUI candidates, 42% improved to become candidates for natural pregnancy. In other words, 16.5%, 30.6%, 38%, and 60% of preoperatively defined IVF-ICSI, IVF, IUI, and natural pregnancy candidates were able to naturally conceive after varicocele repair.

Similarly, Samplaski reported on 373 men who underwent varicocelectomy [18]. TMSC improved for all three preoperatively designated groups (i.e., IVF, IUI, natural pregnancy). Of IVF candidates (TMSC <5 M/ml), 22% became IUI candidates and 32% became natural pregnancy candidates. Of men who were IUI candidates (TMSC 5–9 M/ml), 58% improved to natural pregnancy candidates.

It should be noted that in the Çayan study, 11% of men in couples who were natural pregnancy candidates had worsening of semen parameters, upgrading their need for ART [17]. Additionally, Samplaski et al. reported that 27% of IUI candidates and 17% of natural pregnancy candidates had deterioration in their semen parameters to also indicate a potential need for more intense ART [18]. This being said, Samplaski et al. reported that the rate of deterioration was less than that seen in control men who did not undergo varicocele repair, suggesting that repair may still have benefitted those men by reducing their rate of deterioration.

Recurrent and persistent varicoceles present a unique challenge; however, Çayan and Akbay also reported significant downgrading in the need for ART in this population as well [19]. Specifically, they studied 217 men who had recurrent or persistent varicoceles with either no improvement in semen parameters or failure to achieve pregnancy within 6 months after the original varicocele repair. Of these men, 120 elected to undergo repeat varicocelectomy. Sperm counts increased in the re-operated men and they had higher overall pregnancy rates (59.5% versus 39.2%), higher natural pregnancy rate (39.7% vs 15.8%), and lower need for ART (60.3% versus 84.2%) compared to those who elected not to re-repair their varicoceles. Interestingly, of those who proceeded with ART, 36.8% were able to attain pregnancy with IUI compared to 12.5% of the controls.

Special Considerations

Ultimately, two of the most significant considerations for patients are time and cost. Clearly, to realize the benefits of varicocelectomy would require a delay to the start of ART. While outcomes may improve with varicocelectomy, waiting several more months may not be palatable to

couples that have been trying for several years to conceive or those in which the female partner is pushing the limits of her fertile window. In these cases, proceeding with ART and then performing varicocelectomy may be a viable option to maximize success and minimize treatment time should the initial rounds of ART fail.

There is relatively little research into the cost of varicocelectomy followed by ART. On the surface, the cost of varicocelectomy followed by ART, particularly IVF-ICSI, would be higher than proceeding directly to ART. However, if varicocelectomy could increase the likelihood of IVF success (i.e., decrease the number of cycles needed to obtain a live birth), or if varicocelectomy could reduce the degree of ART needed, then varicocelectomy prior to ART may be more cost effective than going straight to ART.

Dubin and colleagues reported on the cost-effectiveness of varicocelectomy prior to ART in men with severe oligozoospermia (TMSC <2 million) [20]. Like most studies discussed above, the authors reported an overall significant increase in TMSC in the majority of men. Indeed, after surgery, 10 of the 17 men had TMSC >2 million (which would facilitate IUI prior to IVF) and one was able to naturally conceive. Of the 7 men who underwent IUI, 2 were able to conceive. Varicocelectomy followed by IUI was calculated to be significantly cheaper per pregnancy than proceeding straight to IVF-ICSI. Given that many couples need multiple rounds of IVF, Dubin et al. concluded that it was cost effective to perform varicocelectomy first in men with TMSC <2 million.

The cost savings of varicocelectomy prior to ART may be further amplified in couples who desire to have more than one child; however, this remains to be studied. Additionally, varicocelectomy prior to ART may be significantly cheaper to the patient as varicocelectomy may be covered by insurance while IUI and IVF frequently are not.

Limitations

The literature presented has focused on ART outcomes in men who have clinical varicoceles and abnormal semen parameters. A recent meta-analysis of outcomes of subclinical varicocele repair found no significant benefit to ART outcomes [1]. While meta-analyses have not been performed that examine varicocele repair in men with normal semen parameters, the European Association of Urology found Grade A evidence against repair in this group [2].

All the studies to date have been retrospective reviews of the literature and subject to significant bias. Early studies by Ashkenazi and Yamamoto did not include non-varicocelectomy controls [5, 6]. In all the other studies, the decision to undergo varicocelectomy was at the discretion of the patient after discussion with the physician. Thus, there may have been bias that may have lead the men to undergo varicocelectomy. For example, the report by Zini et al. demonstrated that men with worse semen parameters are more likely to undergo varicocelectomy [11]. Additionally, the study by Pasqualotto and colleagues revealed a bias in that men undergoing varicocelectomy had a significantly longer history of infertility than those who did not [10]. Studies in which these specific biases were not present report significant benefit to varicocelectomy prior to ART. Nonetheless, large, prospective, randomized trials would be necessary to better control for bias and determine the true benefit.

While the literature cited above investigates the effects of varicocelectomy on pregnancy rates and live birth rates, there is relatively little literature investigating healthy birth rates and the presence of genetic abnormalities. While there is insufficient evidence to suggest that varicocelectomy improves these outcomes, there is strong evidence that varicocelectomy reduces DNA fragmentation (reviewed in [21]), which theoretically could lead to fewer mutations in the DNA, changes to the sperm epigenome and improved health in the offspring.

Conclusion

The literature and recent meta-analyses show a benefit for varicocelectomy in improving semen parameters, particularly TMSC. While the majority of the literature indicates an improvement in live birth rate

in men with clinical varicocele who undergo varicocelectomy, the greater benefit appears when the entire spectrum of infertility treatment is considered. Decreasing the degree of ART needed for pregnancy and significantly increasing the number of couples able to conceive naturally—combined with better live birth rates when ART is needed—all indicate that varicocelectomy prior to ART should be strongly considered.

Varicocelectomy is not without cost, however. While varicocelectomy prior to ART may increase the cost for an individual patient that ultimately proceeds to IVF-ICSI, the above data point to potential savings to the healthcare system as a whole by decreasing the need for ART and the number of cycles necessary to attain a live birth. Financially, in the United States and Europe, varicocelectomy is often covered by insurance but ART is not. Thus, it may also be cost advantageous to the patients to perform varicocelectomy first. This may be particularly true if the couple desires to have more than one child. The long-term benefit of varicocelectomy on subsequent live birth rates (both natural and assisted) needs further study.

The one cost, however, that cannot be avoided is time. While varicocelectomy has been shown to significantly improve semen parameters, the effect is not immediate and takes a minimum of several months to come to fruition. While this delay may not seem significant on the surface, this delay may not be a cost worth bearing to couples that have been trying to conceive for years and women of very advanced maternal age—particularly if they only desire to have one child. The benefit of varicocelectomy on decreasing the number of IVF-ICSI cycles in this specific population needs further study.

In summary, the repair of clinical varicoceles in men with abnormal semen parameters and infertility appears to decrease the need for ART and improve ART outcomes. Thus, varicocelectomy should be discussed and encouraged in these men prior to initiating ART. Significantly more research is needed, however, to understand the true impacts of varicocelectomy on live birth rates and benefits to the health of the offspring.

Review Criteria

PubMed, Google Scholar, and MEDLINE were searched in May–July 2018, using the following key words: "varicocele," "varicocele repair," "varicocelectomy," "male infertility," "assisted reproductive technologies," "intrauterine insemination," "in vitro fertilization," "intracytoplasmic sperm injection," and "outcomes." All peer-reviewed articles were included that related to the impact of repairing clinical varicoceles on ART outcomes.

Multiple Choice Questions and Answers

1. Improvements in *clinical pregnancy rates* in couples undergoing ART after repair of varicocele in the male partner have been reported for the following group(s):
   (a) Intrauterine insemination
   (b) In vitro fertilization
   (c) IVF-ICSI
   (d) **All of the above**
2. Improvements in *live birth rates* in couples undergoing ART after repair of varicocele in the male partner have been reported for the following groups(s):
   (a) Intrauterine insemination
   (b) In vitro fertilization
   (c) IVF-ICSI
   (d) **All of the above**
3. Based on the current literature, which of the following groups have shown increased rates of natural pregnancy following repair of clinical varicocele?
   (a) Men with non-obstructive azoospermia (NOA)
   (b) Couples who are candidates for IUI
   (c) Couples who are candidates for IVF-ICSI
   (d) **All of the above**
4. When evaluating total motile sperm count before and after varicocele repair, which of the following is NOT true?

(a) Up to 58% of men became candidates for less intensive ART
(b) Up to 27% of men had worsening of their semen parameters
(c) **The rate of deterioration of semen parameters after varicocele repair was worse than in non-treated men**
(d) None of the above

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Varicocele and Azoospermia

25

Parviz Keikhosrow Kavoussi

Key Points

- In some men, varicocele is the exception to testicular etiologies of azoospermia being non-correctable.
- Sperm returns to ejaculate in 10% to 50% of non-obstructive azoospermic men after varicocele repair, mostly with hypospermatogenesis or late maturation arrest, rather than Sertoli-cell only or early maturation arrest.
- Semen samples should be cryopreserved after return of sperm to the ejaculate following varicocele repair as cases of relapse to azoospermia have been reported.
- Data show higher sperm retrieval, fertilization, and pregnancy rates with IVF/ICSI for men who remain azoospermic after varicocele repair, compared to varicoceles left intact in azoospermic men undergoing sperm retrieval.
- In men with varicoceles who desire vasectomy reversal, a recommended approach is to undergo vasectomy reversal, assess semen parameters, and then decide if varicocele repair is indicated subsequently.

Introduction

Approximately 15% of couples in the United States are considered infertile, after being unsuccessful at achieving a pregnancy for 1 year with unprotected intercourse. Male factor is solely responsible for 20% of these cases, while it is a contributory factor in conjunction with female infertility factors in an additional 40%, indicating that 60% of the time that there is difficulty conceiving, there is male factor involvement [1]. One percent of men in the general population are azoospermic and approximately 15% of men presenting for infertility evaluations are found to be azoospermic [2, 3]. The definition of azoospermia is a complete absence of sperm from the ejaculate. Azoospermia is diagnosed by at least two semen analyses revealing a complete absence of sperm in the semen by high power light microscopy as well as revealing no sperm cells in the centrifuged concentrated pellet after centrifugation for 15 min at a speed of at least 3000 × g or greater [4].

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P. K. Kavoussi (✉)
Department of Reproductive Urology, Austin Fertility & Reproductive Medicine/Westlake IVF, Austin, TX, USA

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Men with azoospermia are divided into one of three categories: pre-testicular, testicular, and post-testicular etiologies. Pre-testicular etiologies include hormonal causes that have an adverse impact on spermatogenesis, such as a hypogonadotropic state. Testicular etiologies refer to primary testicular failure of spermatogenesis in the testes. Post-testicular etiologies include obstruction of the reproductive tract and ejaculatory disorders. Although the majority of testicular etiologies of azoospermia are typically not correctable, varicocele is the exception. This is the one opportunity for men with non-obstructive azoospermia to potentially regain sperm in the ejaculate for an opportunity to achieve a pregnancy spontaneously or with assisted reproductive technology without a sperm retrieval.

This chapter aims to discuss the impact of varicoceles on testicular function in men with azoospermia, the impact of varicocele repair on azoospermic men, and the impact of varicocele repair on sperm retrievals and outcomes for the couples when the male partner does not have return of sperm to the ejaculate following varicocele repair (please see Video 25.1 demonstrating subinguinal microsurgical varicocele repair technique). Further discussion includes men who have varicoceles and iatrogenic azoospermia via vasectomies desiring vasectomy reversal, as well as men with varicoceles who are interested in achieving azoospermia via vasectomy (Table 25.1).

Table 25.1 Outcomes of varicocele repair in non-obstructive azoospermic men: percentage of non-obstructive azoospermic men with return of sperm to the ejaculate after varicocele repair, percentage of couples who achieved spontaneous pregnancy after varicocele repair with a preoperative non-obstructive azoospermic male partner, and percentage of couples achieving pregnancy with assisted reproductive technology (ART) after varicocele repair with a preoperative non-obstructive azoospermic male partner

Study	Return of Sperm to Ejaculate	Spontaneous Pregnancy	Pregnancy with ART
Matthews et al.	12/22 (55%)	3/22 (14%)	Not reported
Kim et al.	12/28 (43%)	0	1/28 (4%)
Cakan et al.	3/13 (23%)	0	0/1 (0%)
Ishikawa et al.	2/6 (33%)	Not reported	Not reported
Lee et al.	7/19 (36%)	1/19 (5%)	Not reported
Weedin et al.	91/233 (39%)	14/223 (6%)	Not reported
Youssef et al.	27/83 (34%)	6/83 (7%)	Not reported
Abdel-Meguid	10/31 (32%)	Not reported	Not reported
Aboutaleb et al.	6/20 (30%)	Not reported	Not reported
Alves et al.	5/25 (20%)	Not reported	Not reported

Azoospermia

The evaluation of the azoosperrmic male should include a complete medical history, a physical examination with focus on secondary sexual characteristics and the genitourinary examination. The evaluation should include a history of prior fertility; childhood medical problems such as cryptorchidism or viral orchitis; history of pelvic, testicular, or inguinal surgery; genitourinary infections such as epididymitis or urethritis; heat exposures or fevers; gonadotoxic exposures such as chemotherapy or radiation; and family history of cystic fibrosis, or reproductive issues. The examiner should take note of testicular volumes and consistencies, body habitus, the presence or absence of gynecomastia, hair distribution, the presence or absence of the vasa deferentia, the consistency of the epididymides and whether they are dilated or flat, digital rectal examination assessing for a midline prostatic cyst or palpable seminal vesicles, and the presence of unilateral or bilateral varicoceles. A varicocele is a diagnosis made by physical examination, which should be performed with the patient in both the upright and recumbent positions. Varicoceles disappear or significantly reduce when in the recumbent position. A clinical varicocele is described as feeling like a "bag of worms". Clinically palpable varicoceles are graded on a scale of three. Grade 1: only palpable with Valsalva maneuver, grade 2: easily palpable, and grade 3: visually identifiable varicocele. Laboratory evaluation of the azoospermic male, along with two properly obtained semen analyses, includes a serum hor-

mone evaluation including a testosterone level and follicle stimulating hormone (FSH) at minimum [5, 6].

Further evaluation of the azoospermic male is necessary to diagnose the specific condition resulting in azoospermia. Agenesis or absence of the vasa deferentia is a diagnosis of physical examination. A mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene is the most frequent etiology of congenital bilateral absence of the vas deferens (CBAVD). An identifiable abnormality of the CFTR gene is found in approximately 70% of men with no clinical evidence of cystic fibrosis and CBAVD, and nearly all men with clinical cystic fibrosis have CBAVD [7, 8]. The diagnosis of CBAVD is made by physical examination, imaging and surgical exploration are not necessary. Men with CBAVD and their female partners should undergo CFTR gene testing and genetic counseling [9, 10]. The physical examination finding of bilateral testicular atrophy in the azoospermic male can be an indication of primary testicular dysfunction or secondary testicular failure. Azoospermic men with atrophic testicles, normal or low serum testosterone levels, and elevated FSH levels have findings consistent with primary testicular failure. In such men, further testing should include a karyotype and a Y chromosome microdeletion assay. Azoospermic men with bilateral testicular atrophy, low serum testosterone, low FSH, and low luteinizing hormone (LH) levels are categorized as having secondary hypogonadism, or hypogonadotropic hypogonadism. Men with azoospermia and secondary hypogonadism should be further evaluated with serum prolactin levels and pituitary imaging with MRI, especially when the prolactin is elevated to assess for a functional prolactin-producing pituitary adenoma associated with the hypogonadotropic state [5].

Azoospermic men with normal volume testicles, palpable vasa deferentia, and a low semen volume may have ejaculatory duct obstruction or ejaculatory dysfunction. Azoosperrmic men with normal testicular volumes, palpable vasa deferentia, and a normal semen volume may have either reproductive tract obstruction or testicular spermatogenic dysfunction. Semen volume and FSH levels help differentiate between these etiologies. A diagnostic testicular biopsy is recommended in men with azoospermia with normal testicular volumes, at least one palpable vas deferens, and a normal FSH level [5]. Azoospermic men with low semen volumes (less than 1 ml), a normal testosterone level, with bilateral palpable vasa deferentia, may be azoospermic due to ejaculatory duct obstruction which is assessed by transrectal ultrasound revealing dilated seminal vesicles with anteroposterior diameters of greater than 1.5 cm or a midline prostatic cyst [11–13].

Varicocele and Non-Obstructive Azoospermia

When other etiologies of azoospermia have been ruled out, surgical repair may be considered in men with varicoceles. Varicoceles are abnormally dilated scrotal veins, which are found in approximately 15% of men in the general population and in 40% of men presenting for infertility evaluations, making varicoceles the most common diagnosis made in infertile men [14]. As 1% of men in the general population are azoospermic and 15% of men presenting for infertility are azoospermic, there tends to be a fair amount of overlap in azoospermic men with varicoceles [1, 14]. There are a number of theories of the mechanisms by which varicoceles may adversely impact spermatogenesis and testicular function which may potentially lead to azoospermia. The majority of the data indicate that varicoceles impact testicular function by increasing intratesticular temperatures due to interruption of counter-current heat exchange in the pampiniform plexus with opposing flows in a central arterial system [15–17]. The potential mechanisms of cellular damage from varicoceles that have been proposed include sperm DNA fragmentation, apoptosis, increasing reactive oxygen species through oxidative stress, intracellular ionic and metabolic changes, and predisposition to sperm aneuploidy [18–32]. Men with varicoceles demonstrate higher mean tubular apoptotic indices than controls [33].

An animal study in a rat model supports the hypothesis of retrograde flow of adrenal and renal metabolites worsening varicocele-induced testicular damage [34]. Data have shown that thermal abnormalities and class IIC meiotic abnormalities are reversible after varicocele repair in the testes of men with non-obstructive azoospermia (NOA) when repeat evaluation was performed 6 months after varicocele repair [35].

Although the level of evidence is not definitive, there appears to be a role for varicocele repair in men with NOA with palpable varicoceles. A number of studies have revealed return of sperm to the ejaculate in men with NOA who underwent varicocele repair. Return of sperm to the ejaculate is reported in approximately 10% to 50% of men with NOA post-varicocele repair, the majority of which indicate more favorable outcomes associated with testicular histology of hypospermatogenesis or late maturation arrest, as opposed to less favorable outcomes after repair in men with Sertoli-cell only or early maturation arrest [36–53]. Men who respond can avoid a testicular sperm extraction (TESE) and may use the sperm in the semen with assisted reproductive technology (ART). Testicular histology appears to be the greatest predictor of return of sperm to the ejaculate after varicocele repair. Sertoli-cell only and early maturation arrest patterns result in the poorest response to varicocele repair in azoospermic men. There have not been identifiable predictors for men who will relapse to azoospermia after recovering sperm in the ejaculate following varicocele repair [45, 51, 54, 55]. Seminal plasma micro-RNA (miR)-192a levels have been found to be higher in azoospermic men who did not have return of sperm to the ejaculate after varicocele repair when compared to those that did have return of sperm to the semen after varicocele repair and controls. miR-192a-induced GC-2 cell apoptosis through the activation of Caspase-3 protein may be a useful predictor of response to varicocele repair [56]. Some level of controversy exists regarding varicocele repair in men with NOA due to variable results in studies [57]. The presence of a Y chromosome microdeletion should be assessed prior to varicocele repair, as semen parameter responses are less favorable with varicocele repair when a Y chromosome microdeletion is present [58].

In a study of 33 men with varicoceles and azoospermia, histology of the testes in biopsies revealed Sertoli-cell only in nine of these men while another six men had degenerative changes in the seminiferous tubules of one or both testes. Fifteen men had decreased spermatogenesis on the ipsilateral side of the varicocele, and histology on the remaining men revealed active spermatogenesis with absent spermiogenesis. In all cases the changes were equal or more severe on the ipsilateral side with the varicocele. Twelve men (34%) had return of sperm to the ejaculate within 2–14 months following the varicocele repair [59]. Small series have reported pregnancy rates in azoospermic men treated only with varicocele repair, such as the series by Mehan resulting in pregnancies in two out of 10 azoospermic men treated with varicocele repair [60]. Another small series compared response rates to varicocele repair in men with azoospermia (n = 24) versus virtual azoospermia, which was defined as men who fluctuated between having samples revealing azoospermia and cryptozoospermia (n = 14). With a mean follow up of 14 months after varicocele repair, sperm was identified in the ejaculate of five (21%) of the azoospermic men, three of which had testicular histology revealing maturation arrest at the spermatid stage, one with Sertoli-cell only pattern with focal spermatogenesis, and one with hypospermatogenesis. None of the patients with pure Sertoli-cell only or spermatocyte stage maturation arrest patterns had improvement of azoospermia after varicocele repair. Of the men with virtual azoospermia, 12 (85%) had improvement in semen parameters and four of them (28%) reached a total motile count of more than five million. After repair, three men (21%) were able to achieve a spontaneous pregnancy with the level of improvement [61]. Although varicocele embolization is associated with high rate of failure, in which up to 20% of cases technical problems result in the interventional radiologists' inability to completely access internal spermatic veins to effectively embolize them, and recurrence rates are significantly higher than micro-

surgical repairs, approximately 15% with embolization versus 1%–2% with microsurgical repair, some data is available on azoospermic men [6]. Thirty-two azoospermic men were treated with percutaneous embolization and 18 men (56%) had return of sperm to the ejaculate with a mean sperm concentration which improved from zero to $3.81 \pm 1.69 \times 10^6$/ml [62]. It is recommended that semen samples are cryopreserved after return of sperm to the ejaculate following varicocele repair or embolization, as there have been reported cases of relapse to azoospermia as early as 6 months after recovery of spermatogenesis [41, 48, 63].

Varicocele and Sperm Retrieval

Having sperm return to the ejaculate in NOA men who undergo varicocele repair does not always equate to having adequate sperm for use with in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). In one study, of 32 men with NOA who underwent varicocele repair, seven of them (22%) had sperm identified on a post-varicocelectomy semen analysis; however, only three (9.6%) had adequate motile sperm in the ejaculate for use with IVF/ICSI and were able to avoid TESE [57]. Other data has indicated that varicocele repair in men with NOA improves sperm retrieval rates at the time of TESE and improves IVF/ICSI outcomes. One study revealed a sperm retrieval rate of 60.8% in men who remained azoospermic after varicocele repair versus a sperm retrieval rate of 38.5% in NOA men who did not undergo varicocele repair. The varicocele repair group also had a significantly higher clinical pregnancy rate and live birth rate with IVF/ICSI than the men who did not undergo repair, 74.2% versus 52.3% and 64.5% versus 41.5%, respectively [64]. Another study compared couples who underwent IVF/ICSI when men underwent varicocele repair versus men who left their varicoceles untreated. The sperm retrieval rate with microdissection testicular sperm extraction (microTESE) was significantly higher in the men who previously had their varicoceles repaired, 53% versus 30%. However, there was no difference in fertilization rate, rate of high-quality embryos, or mean number of transferred embryos. The clinical pregnancy rate was significantly higher in the varicocele treated group versus the untreated group, at 31% versus 22%, respectively [65]. Multiple studies have shown similar findings of improved sperm retrieval rates, fertilization rates, pregnancy rates, and live birth rates with microTESE-IVF/ICSI after varicocele repair versus leaving varicoceles intact [52, 66–68]. One study suggested that testicular histology improved after varicocele repair in NOA men. Testicular biopsy was performed in men at the time of varicocele repair and again at the time of microTESE in men who remained azoospermic after varicocele repair. Fourteen men were classified as Sertoli-cell only from the biopsies at the time of varicocele repair and were reclassified as focal spermatogenesis in two of them and late maturation arrest in three of them from the biopsies at the time of microTESE [69]. However, there may be baseline heterogeneity in testicular histology in certain testicular units at baseline.

Varicocele in Men Desiring Vasectomy Reversal

Approximately 500,000 men undergo vasectomy in the United States annually. Ultimately 6% of these men will change their minds and pursue vasectomy reversal [70]. Considering that 15% of men in the general population have a varicocele, there is certainly some overlap in men desiring vasectomy reversal and men with varicoceles [14]. In a properly performed varicocele repair, all spermatic veins are ligated, leaving only the vasal veins as the remaining venous drainage for the testicle (Fig. 25.1). As there is the possibility that vasal veins may have been compromised at the time of vasectomy, and there is potential risk of compromising vasal veins at the time of vasectomy reversal, not performing concomitant varicocele repair with vasectomy reversal will minimize the risk of compromise to the testis due to

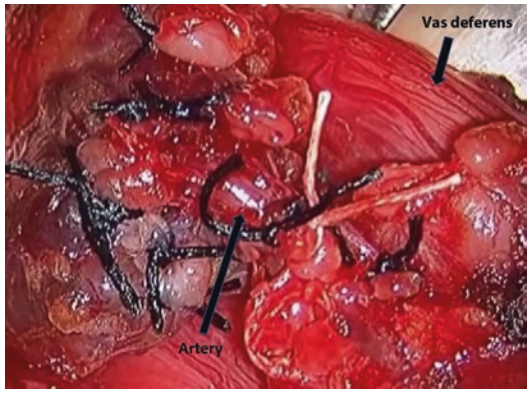

Fig. 25.1 Completed microsurgical subinguinal varicocele repair with all spermatic veins ligated, leaving only the vasal veins as the remaining venous drainage for the testicle. The vasal vasculature can be visualized adherent to the vas deferens. The gonadal artery is preserved

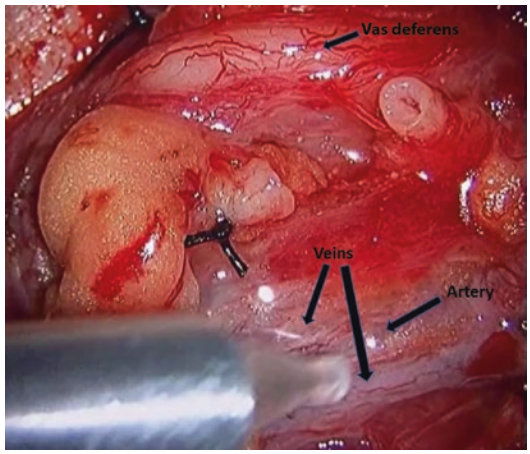

Fig. 25.2 Microsurgical visualization of the gonadal artery with assistance of the microtipped Doppler, with the adherent veins visualized

complete venous ligation. Additionally, the vasal artery which should be preserved during varicocele repair may have been compromised during vasectomy and is at risk for compromise with vasectomy reversal [71]. In one case series, varicocele repair was performed concomitantly with vasectomy reversal, with the technique of leaving the cremasteric veins and the veins adherent to the gonadal artery intact to maintain venous return and to minimize risk to the gonadal artery (Fig. 25.2). Five out of 26 varicoceles (19%) recurred [72]. This is in comparison with an approximately 1% recurrence rate for a microsurgical varicocele repair alone [6]. A different case series evaluated a series of concomitant microsurgical varicocele repairs and vasovasostomies without intentionally preserving the veins adherent to the gonadal artery or the cremasteric veins and reported no incidents of testicular atrophy and revealed a low varicocele recurrence rate [73]. A commonly implemented approach is performing vasectomy reversal and assessing the response with semen parameters, and based on these results, the decision may be made to perform a subsequent varicocele repair when necessary after venous and arterial vessels have formed with neovascularization across the anastomosis [71].

Varicocelectomy and Vasectomy (Men Desiring Azoospermia)

Although varicocele repair is commonly thought of as a surgery to improve fertility, scrotal pain and potential improvement of testosterone levels in hypogonadal men are other indications for varicocele repair. Some men interested in varicocele repair for the indications of pain or hypogonadism are interested in concomitant vasectomy for sterilization. Men undergoing evaluation for vasectomy with asymptomatic varicoceles have their varicoceles diagnosed at a younger age than age-matched controls [74]. A series of 18 men undergoing vasectomy with simultaneous varicocele repair was reported. All men underwent microsurgical subinguinal varicocele repair with vasectomy, ligating all cremasteric, spermatic, and gubernacular veins and the vasectomy was performed under microsurgical magnification to preserve deferential vessels. No cases of vasectomy failures, varicocele recurrences, testicular atrophy, or complications were reported. When vasectomy with concomitant varicocele repair is to be performed, it should be performed microsurgically in order to preserve the gonadal artery and the deferential vessels to minimize the risks of insufficient testicular venous drainage and injury to the testicular arterial supply (Fig. 25.3) [75].

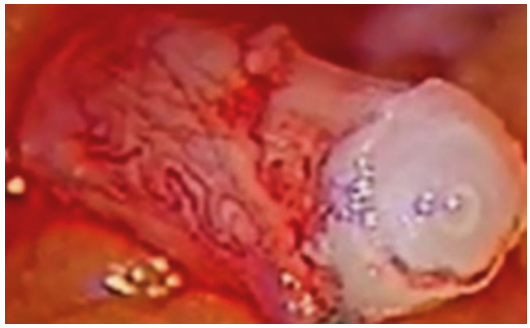

Fig. 25.3 Microsurgical vasectomy with vasal adventitia intact preserving deferential vessels

Conclusion

As 40% of men presenting for fertility evaluations will be found to have palpable varicoceles and 1% of men presenting will be azoospermic, there will be quite a number of patients presenting with varicoceles and azoospermia. It is important to offer the optimal options for treatment for these men. Once a proper evaluation has been completed and other etiologies of azoospermia have been ruled out, varicocele repair may be offered as a first step with a potential to improve spermatogenesis to re-establish sperm in the semen and to optimize sperm retrieval and pregnancy outcomes in those who remain azoospermic and require subsequent sperm retrieval.

Review Criteria

An extensive search of all studies examining the relationship between varicoceles and azoospermia, between varicoceles and sperm retrieval, between varicoceles and vasectomy reversal, and between varicoceles and vasectomy was performed using search engines such as PubMed and Google Scholar. The overall strategy for study identification and data extraction was based on the following key words: "varicocele", "azoospermia", "sperm retrieval", "testicular sperm extraction", "vasectomy reversal" and "vasectomy". Articles published in languages other than English were excluded.

Multiple Choice Questions and Answers

1. What is the one testicular etiology of azoospermia which may be correctable?
   (a) Klinefelter's syndrome
   (b) AZFa Y chromosome microdeletion
   (c) **Varicocele**
   (d) Chemotherapy-related testicular changes
2. What is the histological testicular pattern that is most favorable for return of sperm to the ejaculate in non-obstructive azoospermic men who undergo varicocele repair?
   (a) Sertoli-cell only
   (b) Early maturation arrest
   (c) **Late maturation arrest**
   (d) None of the above
3. Higher sperm retrieval rates are reported in azoospermic men after:
   (a) Leaving varicoceles intact
   (b) **Repairing varicoceles**
   (c) Repairing hydroceles
   (d) Excising spermatoceles
4. The greatest predictor of return of sperm to the ejaculate in azoospermic men following varicocele repair is:
   (a) FSH level
   (b) Testicular volumes
   (c) Testosterone levels
   (d) **Histologic pattern of the testis**
5. The purpose of performing a vasectomy with a microsurgical technique when it is performed with concomitant varicocele repair is:
   (a) To visualize the intraluminal cauterization of the vas deferens
   (b) To ensure a proper tissue interposition between the divided ends of the vas deferens
   (c) To perform the vasectomy with a no-scalpel technique
   (d) **To preserve the gonadal artery and the deferential vessels to minimize the risks of insufficient testicular venous drainage and injury to the testicular arterial supply**

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26

Prognostic Factors for a Favorable Outcome After Varicocele Repair in Adults with Infertility

Adit Shah and Mary K. Samplaski

Key Points

- The effect of varicocele repair on male fertility remains controversial. Not all men with varicoceles will experience subfertility, and not all men undergoing varicocele repair will have an improvement in their fertility after varicocele repair.
- Men with higher-grade varicoceles will have larger improvements in their post-repair semen parameters.
- A higher baseline sperm concentration or total motile sperm count (TMC) is predictive of a larger post-repair TMC and also seems to correlate with pregnancy outcomes.
- Varicocele repair does seem to restore sperm to the ejaculate in 20–40% of men with nonobstructive azoospermia, but the factors predicting for this are still being determined.
- Genetics will likely play an increasing role in the future in predicting which males will respond favorably to varicocele repair.

Introduction

The effect of varicocele repair on male fertility remains controversial. Both the American Urological Association (AUA) and the American Society for Reproductive Medicine (ASRM) currently recommend varicocele repair for infertile men with a clinical varicocele and one or more abnormal semen parameters [1]. However, not all men with varicoceles will experience subfertility. Illustrating this, a study of 598 men with proven fertility seeking a vasectomy found that 16% of these men had a varicocele [2]. In addition, 45–65% of men with varicoceles will have completely normal semen parameters [3]. It is estimated that in subfertile men with a clinical varicocele, in 40% of these men, the varicocele will be a contributing factor. This increases to 80% of men experiencing secondary infertility. Furthermore, not all men with varicoceles will have an improvement in their fertility after varicocele repair.

Most men with abnormal semen parameters will experience some degree of improvement after a varicocele repair. A recent meta-analysis by Schauer et al. looked at the impact of three surgical techniques (high ligation, inguinal varicocelectomy, and the subinguinal approach) for varicocelectomy on sperm parameters (count and motility) and pregnancy rates. All three surgical approaches led to significant postoperative improvement in both semen parameters and preg-

A. Shah · M. K. Samplaski (✉)
Institute of Urology, University of Southern California, Los Angeles, CA, USA
e-mail: mary.samplaski@med.usc.edu

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nancy outcomes [4]. Another meta-analysis by Baazeem et al. aggregated data from four randomized controlled trials reporting on pregnancy outcomes after the repair of clinical varicoceles. They found that combined improvement in sperm concentration was 12.32 × 10^6/mL ($p < 0.0001$), and combined improvement in sperm total and progressive motility were 10.86% ($p < 0.0001$) and 9.69% ($p = 0.003$), respectively. They also found that surgical intervention reduced sperm DNA damage and seminal oxidative stress and improved sperm ultramorphology [5].

While we have an abundance of data looking at the various technical aspects of varicocele repair, pre- and post-repair semen parameters, and even reproductive outcomes after repair, we do not yet have predictors for which men will benefit the most from having a surgical repair performed in the first place. Not only does this hinder our ability as practitioners to appropriately counsel our patients, it also prevents us from identifying underlying factors that may help us better understand the pathophysiology underlying the association between varicoceles and infertility. This knowledge has the potential to improve our surgical technique and may even help uncover alternative therapeutic modalities. Interestingly, recent data have even suggested that men who do not respond to varicocele repair may have a different underlying genetic fingerprint compared with men who do [6].

The seminal study seeking to identify predictive factors for reproductive improvement after varicocelectomy was performed by Marks et al. in 1986, looking at 130 men with oligozoospermia and clinical varicoceles [7]. They compared men that underwent microsurgical repair with a control group that was treated with clomiphene citrate, the primary outcome being pregnancy rates. Four variables (testicular length >4.5 cm, sperm density >50 × 10^6/mL, sperm motility >60%, and serum follicle-stimulating hormone (FSH) <300 ng/mL) were found to be predictive of postoperative success. Interestingly, varicocele grade was not predictive of postoperative pregnancy. More recently, a prospective Canadian study of 123 subfertile patients undergoing microscopic subinguinal varicocelectomy found that baseline varicocele grade 2 or 3, sperm density >8 × 10^6/mL and progressive sperm motility >18% were independent predictors of semen parameter improvement after varicocelectomy [8].

This chapter reviews the current literature regarding prognostic factors for favorable outcomes after varicocele repair in infertile adults. This would obviously be useful to target efforts directed at these patient populations. In the most comprehensive review on this topic, we performed a systematic review of the literature, looking at factors that may predict for an improvement in reproductive outcomes after varicocele repair. While most of the published data has semen parameters as the outcome, the majority of men undergo varicocele repair in hopes that it will help them achieve pregnancy, either naturally or through assisted reproductive technologies (ART). Therefore, within each section, we grouped reproductive outcomes into semen parameters and pregnancy rates (either assisted or natural). Data for all of these outcomes was not available for all of the preoperative factors. A summary of these findings can be found in Table 26.1.

Unilateral or Bilateral Repair

While most varicoceles are left-sided, there are some men who will have either clinical or radiographic right-sided varicoceles. The role for bilateral repair in these men is not completely clear. There have been several studies looking at this specifically. At present, even though the data is conflicting, the AUA and ASRM currently recommend varicocele repair only for men with clinically palpable varicoceles [1]. Individual surgical decisions may be made on a case-by-case basis based on unique provider experiences and patient circumstances.

Clinically Palpable Left and Subclinical Right Varicoceles

For patients with a clinically palpable left and subclinical right varicocele, the data is slightly conflicting, but overall does support an improvement in semen parameters [9, 10]. Recently, there was a prospective randomized control study of

Table 26.1 Summary of factors that have been shown to be prognostically significant for reproductive outcomes after varicocele repair

Prognostic factor	Finding(s)
Unilateral or bilateral repair	Clinically palpable left and subclinical right varicoceles: Mixed data, but overall suggests mild increase in semen parameters [9, 10] Clinically palpable left and right varicoceles: Data suggests an increase in both semen parameters and pregnancy rates [13, 14]
Varicocele grade	Clear difference in both semen parameters and pregnancy rates correcting grade 2–3 vs. grade 1 varicocele [17–20]
Pre-repair semen parameters	Clear difference in both semen parameters and pregnancy rates in those patients with higher TMC and sperm concentration (specifically when $\geq 5 \times 10^6$/ml) [7, 17, 21, 22]
Nonobstructive azoospermia	Possible benefit in IVF success rates (possible role for genetic analysis to stratify this subgroup moving forward) [6]
Hormones	Limited data showing prognostic utility of low baseline FSH, percentage change in FSH, and high baseline testosterone (no studies looking at LH, estradiol, or prolactin) [7, 23, 29]
Age	Mixed data with studies showing larger degree of improvement in semen parameters in younger men undergoing intervention [31] and another study showing increased natural pregnancy rates in older men who underwent varicocelectomy [32]
Testicular volume	Data to this point had shown no impact [23, 26]; however, new data may point to testicular volume >29.6 ml as a prognostic factor for improvements in semen parameters in both primary and redo varicocele repair [30, 35]
BMI	No identifiable prognostic impact [23, 30]
Pre-repair vein imaging	No identifiable prognostic impact of either vein size or the number of veins ligated intraoperatively [30, 41]
DNA fragmentation	One study showing an improvement in the DNA fragmentation index post varicocelectomy while a higher pre-repair DNA fragmentation index was associated with a larger decrease in postoperative DNA fragmentation [55]
Histology	Multiple studies showing that maturation arrest (late better than early) or hypospermatogenesis has a better prognosis than those with Sertoli cell only [6, 26, 43]
Genetics	Still evolving understanding of chromosomal alterations, genetic polymorphisms, and epigenetic modifications in varicoceles and how they contribute to the variability in the association between varicoceles and fertility

358 subfertile men with left clinical and right subclinical varicoceles undergoing unilateral versus bilateral microsurgical repair. The authors found that while both groups had improvements in semen parameters at 1 year, the bilateral repair group had a larger degree of improvement. In addition, pregnancy rates were higher in the bilateral repair group (42.5% versus 26.0%) [9]. Similarly, a prospective study of 145 men found that men undergoing bilateral repair had greater improvements in sperm concentration (15×10^6/mL to 2×10^6/mL, compared with 15.1×10^6/mL to 21×10^6/mL), motility, and higher natural pregnancy rates (61.6% versus 31.9%) [10].

Conversely, a 2009 study of 104 men with left clinical and right subclinical varicoceles treated with a retroperitoneal approach (unilateral versus bilateral) found no significant difference in the postoperative semen parameters, testicular volume, serum testosterone level, and natural pregnancy rates when the right subclinical varicocele was treated [11]. Finally, a recent meta-analysis of 13 studies and 1357 men sought to determine if there is a role for repair of subclinical varicoceles. The authors found that while surgical correction of subclinical varicocele was associated with a minor increase in sperm density and total motile sperm count (TMC), there was no difference in pregnancy rates [12].

Clinically Palpable Left and Right Varicoceles

For patients with clinically palpable bilateral varicoceles (even when the smaller side is only grade 1), the data indicates that improved semen parameters and pregnancy rates are seen [13, 14]. The

largest study looking at men with bilateral clinically palpable varicoceles (clinical grade 2 or 3 left varicocele and grade 1 right varicocele), undergoing unilateral versus bilateral repair, was from 2006. One hundred and fifty-seven men underwent bilateral and 212 men underwent unilateral left varicocele repair. Semen parameters improved postoperatively in both groups, but the degree of improvement was greater in the bilateral group. In addition, the natural pregnancy rate was higher in the bilateral repair group (49% versus 36%) [14]. Similarly, a prospective study from 1999 looked at 91 men with bilateral clinically palpable varicoceles (left > right), of which 65 underwent bilateral and 26 underwent unilateral left repair. Men undergoing bilateral repair had a larger improvement in sperm concentration, 95.8% in the bilateral repair group compared with 42.6% in the unilateral repair group [13].

Conversely, a study on the same population with bilateral clinically palpable varicoceles (left > right) found no differences in postoperative semen parameters (concentration, motility, morphology) between groups [15]. Another study in this population (65 patients), using the laparoscopic approach for clinically graded varicoceles, also found no differences in post-repair semen parameters (36.52×10^6/mL versus 23.19×10^6/mL) [16].

Varicocele Grade

One factor that has emerged as being relevant more consistently than some others is clinical varicocele grade (based on a physical examination and classified as defined by Dubin and Amelar into grades 0–3). Most studies demonstrate more substantial improvements in both semen parameters and pregnancy outcomes when correcting larger grade varicoceles as compared with smaller grade varicoceles [17–20]. The most recent study on this was by Samplaski et al., looking at 373 subfertile men undergoing varicocele repair. While this study was not specifically focused on varicocele grade, the authors found that larger varicocele grades were associated with larger improvements in semen parameters [17]. Another large series, specifically looking at the role of patient age and varicocele grade on left-sided varicoceles, found that improvements in sperm concentration and motility were greatest after the repair of grade 2 and 3 varicoceles (versus grade 1) (11.04×10^6 sperm/mL versus 12.23×10^6 sperm/mL) [18]. Several other smaller series have also demonstrated this association. One of these looked at 40 men with clinical or radiographic varicoceles treated with varicocelectomy and found that greater improvements in sperm density were seen with increasing grades of varicocele [19]. Finally, there was an early study of 86 subfertile men, who were grouped specifically by grade of their varicoceles. Men with larger varicocele grades had poorer semen parameters at baseline and had larger improvement after repair. Fertility index improved after varicocelectomy in all men, but to a greater degree with larger varicoceles: grade 3 varicocele improved by 128%, grade 2 improved by 21%, and grade 1 improved by 27% [20].

Pre-repair Semen Parameters

The success rate of conceiving, regardless of the conception method used, has been linked to the degree of oligoasthenozoospermia by a number of studies [21]. From this data, threshold levels of total motile sperm counts have been defined for moderate oligoasthenozoospermia (20 million) and severe oligoasthenozoospermia (5 million) [21]. These levels can be used in the clinical counseling of patients undergoing ARTs. But while there is ample data looking at the improvement in semen parameters after a varicocelectomy, a few studies have looked at preoperative semen analysis values and whether they have a prognostic role in predicting post-varicocele repair success. Marks et al. initially commented on the predictive value of both preoperative sperm density and motility relative to the success of a varicocele repair [7]. Logically, a man with higher pre-intervention sperm counts would be expected to have higher post-surgical counts, and this association has been confirmed in multiple studies. The nomogram work by Samplaski et al. found that both post-repair sperm concentration and TMC were correlated with pre-repair sperm concentration [17]. Likewise, a 2014 study

specifically looking at prognostic factors found that age and preoperative sperm density were favorable prognostic factors for post-varicocelectomy success. In this study, a preoperative sperm density of 12×10^6/mL was able to predict successful varicocelectomy with a sensitivity of 77.6% and a specificity of 77.4% [22]. Finally, a retrospective review by Matkov et al. found that men with mild to moderate oligoasthenozoospermia (TMC > 5×10^6 sperm/mL) had significantly better seminal improvement following varicocelectomy as compared with men having baseline TMC >5×10^6 sperm/mL. In addition, men who achieved a postoperative TMC > 20×10^6 were more likely to achieve conception by less invasive techniques (natural pregnancy and intrauterine insemination (IUI) versus in vitro fertilization (IVF)) [21].

Regarding pregnancy outcomes, there was a recent prospective cohort study of 110 men undergoing microsurgical varicocelectomy, looking at pregnancy rates within 2 years of follow-up. On Cox regression analysis, higher baseline TMC and lower follicle-stimulating hormone (FSH) were predictive for spontaneous pregnancy [23]. Likewise, in an early manuscript, Marks et al. found that a pre-repair sperm concentration of >50×10^6 per ejaculate was predictive of a post-repair pregnancy [7]. Prior to this, Zini et al. retrospectively reviewed 159 couples with the male undergoing varicocele repair, looking at assisted and unassisted pregnancy rates. Higher pregnancy rates were seen when the male baseline sperm concentration was ≥5×10^6/ml (61% versus 8%) [24]. Finally, in a study of 242 men with varicoceles undergoing intracytoplasmic sperm injection (ICSI), of whom 80 had their varicoceles repaired before ICSI, the clinical pregnancy rate (60.0% versus 45.0%) and live birth rate (46.2% versus 31.4%) after ICSI were higher in men who had undergone a varicocelectomy than the nonsurgical control group [25].

Nonobstructive Azoospermia

Recently, there have been a series of studies looking at the role of varicocele repair in the nonobstructive azoospermic (NOA) male. While there is limited data, there are several studies that show that varicocele repair in these men may result in the resolution of sperm to the ejaculate, to be used with IVF [6, 26]. Clinical varicocele has been implicated as a cause of testicular dysfunction and infertility in up to 13% of azoospermic men [27]. Converting these men from an azoospermic to severe oligospermic state via varicocele repair may allow some of these men the option to become fathers with IVF. In a 2017 publication looking at 83 men undergoing varicocele repair for NOA with a clinically palpable varicocele, 24% of men had some degree of sperm recovery within 12 months of varicocele repair, including 1/43 (2%) with Sertoli cell only, 10/27 (37%) with maturation arrest, and 9/13 (69%) with hypospermatogenesis. Transcriptome analysis found a distinct difference in the transcription of cell cycle regulation genes between varicocelectomy responsive and nonresponsive patients [6]. An earlier study looking at the role of varicocele repair in NOA found that after varicocele repair, sperm was recovered in 10/31 men. Recovery was associated with baseline histologic pattern, specifically hypospermatogenesis and late maturation arrest [26]. It will be interesting to see if genetics plays a growing role in this population to predict which men have the genetic profile to predict response to varicocele repair.

Hormones

The limited data on the effect of pre-surgical hormone levels on the success rates of varicocelectomy has been mixed. There are a few studies demonstrating a prognostic role for FSH [7, 23, 28], percentage change in FSH [29], and high baseline T [28, 30], but no studies looking at estradiol, leutinizing hormone (LH), or prolactin. In theory, baseline hormones would serve as a surrogate for baseline testicular function or dysfunction. However, whether lower or higher gonadotropins and testosterone values are better predictors is yet to be determined. Marks' 1986 study sparked the interest in the role of pre-repair hormonal levels when they found that FSH <300 ng/ml was a preoperative predictor of postoperative pregnancy [7].

Recent varicocele prognostic data from a series of 120 men reviewed a variety of factors to determine their impact on varicocele repair success, including age, male body mass index (BMI), female BMI, preoperative semen parameters, hormone levels, testicular volume, and grade and side of varicocele. The authors found that baseline TMC and lower baseline FSH were predictive for post-repair natural pregnancy [23]. Conversely, a retrospective review of 97 men undergoing microsurgical left or bilateral inguinal varicocele repair found that on logistic regression analysis, lower pre-operative FSH (3.38 mg/ml) and high testosterone (624 ng/dl) were predictors for sperm concentration improvement [28]. Other studies have not identified a relationship between pre-repair testosterone and reproductive outcomes after varicocele repair [30]. Finally, a 2015 study looking at the prognostic value of a host of pre-repair parameters LH, FSH, total and free testosterone, testis volume, age, testicular pain, BMI, change in FSH, varicocele grade) for post-repair semen parameters found that on multiple regression analysis, only change in FSH and age were predictive [29].

There have been no studies identifying LH, estradiol, or prolactin as being predictive after varicocele repair. Based on this limited data, low baseline FSH or percentage change in FSH and high baseline testosterone are the only hormones with even limited data correlating with reproductive improvements after varicocele repair.

Age

Data are conflicting on the effect of age on varicocele repair outcomes. One study has found that younger men will have greater improvements in seminal parameters [31], and another study found increased pregnancy rates in older men undergoing varicocelectomy [32]. In a prospective study, looking at 67 men presenting with grade 3 varicoceles and infertility, primarily focusing on the impact of age on post-varicocelectomy semen outcomes, age was found to be inversely associated with improvements in semen parameters. While all groups had seminal improvements, patients aged <25 years showed the greatest increase in sperm counts, normal morphology, and motility following varicocelectomy [31]. Similarly, Huang et al. found that age, with an odds ratio of 0.56 (95% confidence interval: 0.41–0.76), had a significant unfavorable association with the likelihood of improvement in semen analysis parameters after varicocelectomy [22]. Finally, a recent review of 100 men undergoing microsurgical varicocelectomy found that postoperative improvements in sperm concentration and motility were greater in men aged <37 years than those aged >37 years [33].

Conversely, in the NOA population, Chen and Chen found that patient age at the time of repair was not associated with the odds of recovery of sperm in the ejaculate following the varicocelectomy [30]. In addition, Zini et al. found no difference in semen parameters or in natural pregnancy rates after varicocelectomy in couples with advanced paternal age (≥40 years) compared with younger age (49% versus 39%, respectively). However, the natural pregnancy rate in couples with advanced paternal age (≥40 years) who underwent varicocelectomy was greater than that of the age-matched control group who did not undergo surgery (49% versus 21%, respectively) [32]. In a large series of >350 men, Samplaski et al. found that older age was related to lesser improvement in post-varicocelectomy sperm motility and morphology, but was not related to improvements in sperm concentration or TMC [34].

Testicular Volume

While older data did not identify a prognostic role for testicular volume in varicocele repair outcomes [23, 26], newer data has found that total testicular volume >29.6 ml is predictive [30, 35]. As testicular volume is generally correlated with male fertility potential, it would follow that this might be correlated with male fertility potential after varicocele repair. In the adolescent population, varicocele repair has been shown to reduce testicular hypoplasia [36]. However, this relationship is not as clear in the adult population. Multiple studies have not identified a prognostic relationship between testicular volume and fertility outcomes

[23, 26]. However, a pair of manuscripts by Chen et al., of 35 subfertile males, found that testicular volume >29.6 ml predicts for improvements in semen parameters in both primary varicocele repair and redo varicocele repair [30, 35].

Body Mass Index

While theoretically higher BMI could transmit higher intraabdominal pressures to the pampiniform plexus, there is scant data on if this affects varicocele repair outcomes [23, 30]. Surprisingly, the available data on this is conflicting. Older studies have shown that higher BMI is associated with a lower risk of having a varicocele [37, 38], while more recent data show that increasing BMI is associated with larger spermatic vein diameters [39]. However, the studies looking at prognostic factors for successful outcomes after varicocele repair have not identified BMI as being prognostically significant for natural pregnancy or semen parameters [23, 30].

Pre-repair Vein Imaging

Logically, it would make sense that larger size veins or a larger number of dilated veins could cause more pathology, which therefore could result in larger improvements after repair. In addition, vein size should theoretically correlate with clinical varicocele grade. However, neither vein number or size has been shown to be consistently prognostic for reproductive outcomes [40, 41]. However, the only study looking at ligated vein size, comprised of 42 men undergoing left-sided microsurgical subinguinal varicocelectomy, found no correlation between ligated vein size (individual or cumulative) and improvements in semen parameters [40].

While few studies have looked specifically at the number of veins ligated, there have been two studies correlating the number of veins ligated at varicocelectomy with postoperative seminal improvements. In the first, men were grouped by the number of veins ligated: ≤5 veins, 6–10 veins, and >10 veins. Only men with >10 veins ligated had improvements in sperm concentration post repair. Of note, all men showed a decrease in FSH levels after repair [42]. Similarly, another prognostic study did find that more veins ligated predicted for greater improvements in semen parameters, with men showing improvements having a mean 9.3 veins ligated and men not showing improvements having 7.9 veins ligated [30]. In contrast, a recent study of 378 men, grouped men by the number of veins ligated, <5, 5–10, and >10 veins. While all men had an improvement in semen parameters, this improvement did not correlate with the number of veins ligated intraoperatively [41].

Histology

There have been several papers looking at the role of histology and the baseline testicular environment, as a possible predictive factor for varicocele repair improvements. All of these to date are looking in the NOA population. In summary, the data seem to show that maturation arrest at a later stage has a greater likelihood for restoration of sperm to the ejaculate as compared with earlier maturation arrest [6, 26, 43]. A recent review and meta-analysis of this found that varicocelectomy in patients with NOA and clinical varicocele is associated with improved surgical sperm retrieval and that approximately 44% of the treated men will have enough sperm in the ejaculate to avoid surgical sperm retrieval [44]. The earliest of these was a 2010 meta-analysis of 223 patients. Post varicocele repair, 39.1% of patients had motile sperm in their ejaculate, and success rates were higher in men with maturation arrest (late better than early) (42.1%) or hypospermatogenesis (54.5%) than those with Sertoli cell only (11.3%) [43]. A subsequent study of 31 men found that 32.2% of men had sperm recovery (this was durable in 19.4%). Sperm were recovered in men with hypospermatogenesis (53.8%) and late maturation arrest (50%), but not early maturation arrest or Sertoli cell only. Histology was the only variable that correlated with sperm recovery [26]. Finally, in a 2017 study of 83 men with NOA, sperm recovery was found in 20 patients (24%), including 1/43 (2%) with Sertoli

cell only, 10/27 (37%) with maturation arrest, and 9/13 (69%) with hypospermatogenesis [6].

Factors Predicting for Natural Pregnancy and/or ART Success Rates

While most of the available literature is directed at semen parameter outcomes, there have been a few studies looking only at factors prognostic for pregnancy. A retrospective review of 610 couples, looking at pregnancy rates and ART utilization in groups of men who did or did not undergo varicocelectomy for clinical varicoceles, was published in 2008. The authors found no difference in natural pregnancy (39% for varicocele repair versus 32% for no varicocele repair) and overall pregnancy (natural and ART) (53% for varicocele repair versus 56% for no varicocele repair) rates, suggesting no difference for couples in which the man underwent varicocele repair versus observation [45]. Another study of 547 couples found that natural pregnancy rates in men undergoing varicocelectomy were inversely correlated with the duration of infertility. Post varicocelectomy, couples with a duration of infertility of 0–3 years had a natural pregnancy rate of 43.9%; 3–6 years, 38.6%; 6–9 years, 38.3%; and > 9 years, 31.7% [46].

Finally, there has been one study looking at the effect varicocelectomy in couples where the female partner is of advanced female age (>35 years) on natural or ART pregnancy rates. Couples with the male undergoing varicocelectomy had similar pregnancy rates (natural pregnancy rate 35% and overall pregnancy rate 41%) compared with couples not undergoing varicocelectomy (natural pregnancy rate 25% and overall pregnancy rate 41%) [47].

Genetics

The role of an individual's genetics in the role of varicocele-induced damage, or the response to repair, is compelling. Some data have found an association between varicoceles and various single genetic polymorphisms [48], but chromosomal alterations and genetic and epigenetic modifications may also play a role in varicoceles and their repair [49]. Of note, there is only a scant data looking at any genetic factors as a prognostic factor for improvements in semen parameters after varicocele repair [6, 49].

DNA Fragmentation

Sperm DNA damage plays a major role in male infertility, miscarriages, abnormalities in the offspring, and failures in assisted reproduction cycles and has a multifactorial etiology. Environmental factors (smoking, chemoradiation, etc.) and failure in the spermatogenesis process have been linked with increased DNA fragmentation, and varicoceles are thought to contribute to this process through oxidative stress. While we have prospective data that in men with elevated sperm DNA fragmentation, varicocele repair will improve this [43], and several recent reviews have confirmed the effectiveness of varicocelectomy as a means of both reducing oxidatively induced sperm DNA damage and potentially improving fertility [44, 45], there is limited data on factors predicting for an improvement in DNA fragmentation after varicocelectomy. To date, there has only been one retrospective study looking at this. Using the sperm chromatin structure assay, post varicocelectomy, there was an improvement in the semen parameters and DNA fragmentation index (42.6% to 20.5%). A higher pre-repair DNA fragmentation index was associated with a larger decrease in postoperative DNA fragmentation index [46].

Somatic/Sperm Chromosomal Alterations

Both microdeletions and chromosomal abnormalities have been linked to varicoceles; however, a cause and effect relationship remains unclear. In a study by Rao et al., chromosomal abnormalities were found in 19.3% of men with varicoceles, including inversions on chromosomes 9 and 2, translocations between chromosomes 4 and 15, a deletion in chromosome 4, and insertion on chromosome 9. These authors also found that 5.3% of patients with varicoceles had microdeletions in sY153, sY158, and sY254 regions of the Y chromosome [50]. Looking at

the chromosomal structure of sperm themselves, Baccetti et al. found that the mean frequency of disomy and diploidy of the sex chromosomes in sperm samples of 46,XY patients with varicocele was higher than that in samples from the control group [51]. However, there are no studies looking at the effect of varicocele repair on chromosomal alterations.

Gene Expression

Due to the relatively large amount of messenger RNA in sperm, there have been several studies looking at differential gene and protein expression patterns in patients with varicoceles. A study by Lima et al. [52] found a lower expression of HSPA2 (heat shock protein A2) gene in adolescents with varicocele and oligozoospermia compared with those with varicocele and normal sperm counts. Mostafa et al. [53] identified increased BAX gene and decreased BCL2 protein levels (both of which are important regulators of apoptosis) in patients with varicocele. These gene and protein abnormalities were associated with decreased sperm concentration, motility and morphology, and overall semen quality [54].

Conclusions

It can be difficult to tease out the individual role of varicocele repair on male fertility, since our patients often modify a variety of factors when they are trying to achieve a pregnancy (lifestyle, behavioral, pharmaceutical, nutraceutical, etc.). However, the available literature does support the role of varicocele repair in increasing male fertility potential. The literature indicates that men with larger grade varicoceles will have larger improvements in their semen parameters. In addition, a higher baseline sperm concentration or TMC is predictive of a larger post-repair TMC and also seems to correlate with pregnancy outcomes. With respect to reproductive outcomes, higher baseline sperm concentration does seem to predict for natural pregnancy or ART pregnancy rates after varicocele repair. In addition, varicocele repair does seem to reduce the need for more invasive modalities of ART. Genetics will likely play an increasing role in predicting which males will respond favorably to varicocele repair.

Review Criteria

An extensive search of studies examining prognostic factors for a favorable outcome after varicocele repair in adults with infertility was performed using the search engine PubMed. The start and end dates for these searches were January 1980 and May 2018, respectively. The overall strategy for study identification and data extraction was based on the following keywords: "varicocele," "varicocele repair," "sperm," "infertile men," "varicocelectomy," "infertility," "semen parameters," "age," "varicocele grade," "bilateral varicocele," "vein size," "FSH," "LH," "testosterone," "estradiol," "hormones," "nonobstructive azoospermia," "testicular volume," "body mass index," "histology," "DNA fragmentation," and "pregnancy rate." Only articles published in English were considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included.

Multiple Choice Questions and Answers

1. What percentage of men with varicoceles will have abnormal semen parameters?
   (a) 10–25%
   (b) 25–45%
   (c) **45–65%**
   (d) 65–85%
   (e) 85–95%
2. What factors have been shown to be predictive of sperm in the ejaculate after varicocele repair in men with nonobstructive azoospermia?
   (a) **Histology**
   (b) Testicular volume
   (c) Baseline FSH
   (d) Number of veins ligated

(e) No factors have been shown to be predictive in this population

3. Which factors have been shown to correlate with pregnancy after varicocele repair?
   (a) Baseline FSH and testicular volume
   (b) Baseline sperm concentration and normal morphology
   (c) Baseline sperm concentration and DNA fragmentation index
   (d) Number and size of veins
   (e) **Baseline sperm concentration and duration of infertility**
4. Which factor has been shown to predict for improvements in sperm DNA fragmentation in men with varicoceles?
   (a) Baseline DNA fragmentation index
   (b) **Varicocele grade**
   (c) Male age
   (d) Number of veins ligated
   (e) Operative time
5. Repair of which grade of clinically palpable varicoceles has been shown to improve semen parameters in subfertile males?
   (a) Grade 1
   (b) Grade 2
   (c) Grade 3
   (d) **Grades 1, 2, and 3**
   (e) Grades 2 and 3 only
6. Which hormones have been consistently shown to be predictive for improvements in semen parameters in men with clinical varicoceles and subfertility?
   (a) FSH
   (b) Total testosterone
   (c) Estradiol
   (d) A and B
   (e) **None**

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Management of Recurrent Varicocele

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Giorgio Ivan Russo and Ege Can Serefoglu

Key Points

- Recurrence of varicocele is not uncommon despite the success of varicocele repair in the literature.
- Missed smaller internal spermatic, external gonadal (cremasteric), and/or external spermatic veins during varicocelectomy may be responsible for varicocele recurrence.
- Surgical correction of recurrent varicocele can be performed with open varicocelectomy, laparoscopic varicocelectomy, or embolization.
- The treatment choice should be based on patients' characteristics together with their preferences in terms of hospital stay, pain, and discomfort.
- Unfortunately, no conclusions can be made regarding seminal parameter recovery or fertility rate after the correction of recurrent varicocele.

Introduction

Varicocele is defined as the dilation of the veins of the pampiniform plexus. It has been reported that at least 15% of adolescent males [1] and more than 30% of infertile men suffer from varicocele [2, 3]. Numerous well-designed studies demonstrated that varicocele repair is associated with improved sperm quality and quantity, which increases the chances of achieving pregnancy among men with abnormal sperm parameters [4, 5]. However, varicocelectomy does not ameliorate sperm parameters in every varicocele patient. Almost half of the men who underwent varicocelectomy will benefit from this surgery and achieve spontaneous pregnancy, whereas the remaining patients will still require assisted reproductive technology (ART) [6–8].

There are several alternatives for the surgical correction of varicocele including open surgical, laparoscopic, or percutaneous approaches. However, small veins can become dilated due to the altered venous circulation after the surgery and varicocele may recur in some cases. Varicocele recurrence rates vary widely depending on the age of the patients, indications for the varicocele treatment, severity of the varicocele, duration of follow-up, and the definition of recurrence being applied [9].

Surgical method used for the correction of the initial varicocele may also have an impact on the recurrence rates. Recurrence after retroperito-

G. I. Russo
Department of Surgery, Urology Section, University of Catania, Catania, Italy

E. C. Serefoglu (✉)
Department of Urology, Bahceci Health Group, Istanbul, Turkey
e-mail: eserefoglu@bahceci.com

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neal high ligation (Palomo) technique is between 7% and 35% [10–14], whereas it is slightly less when the surgery is performed with laparoscopy (between 2.2% and 7.1%) [11, 15–22]. Recurrence rates after macroscopic inguinal or subinguinal approach are also reported to be high (between 0% and 37%) [13, 19–21, 23, 24]; however, adoption of microsurgical approach seems to significantly decrease these rates to 0–3.57% [10–12, 14, 19–21, 24–31]. Unfortunately, recently developed interventional radiology techniques did not reduce the varicocele recurrence as several studies reported recurrence rates between 2% and 25% after radiologic embolization [13, 32–35]. Considering the findings of these series, recent meta-analyses concluded that recurrence after varicocele is lower after open microsurgical inguinal or subinguinal varicocelectomy which is due to the possibility of visualization and ligation of all spermatic veins with higher magnification [21, 36].

Of note, the experience of the clinicians plays an important role in the palpation of the recurrent varicoceles and consequently may affect the recurrence rates. There may be variations in the accuracy of the clinical diagnosis of varicocele recurrence among physicians with different levels of training [37]; thus, some authors advocated the use of ultrasound after varicocele repair for more accurate diagnosis of the recurrence [38]. However the experience of the sonographer in detecting small veins after varicocele surgery and the clinical relevance of diagnosing subclinical varicocele recurrences with ultrasound or venography remain to be controversial.

Etiology of Recurrent Varicocele

The causes of recurrent varicocele are not clear. Although increased duration of venous reflux (>4.5 seconds) of the pampiniform plexus veins [39] and lesser body mass index (<25 kg/m^2) [40] are found to be associated with increased recurrence rates after varicocele operation, surgical technique is the most commonly cited contributing factor to recurrence. The recurrences after macroscopic inguinal or subinguinal varicocelectomy may be due to the missed smaller internal spermatic veins, which later dilate due to the altered venous flow. On the other hand, the higher recurrence rates seen after the high ligation techniques (retroperitoneal or laparoscopic approaches) may be the consequence of the inability to ligate external gonadal (cremasteric) vessels or the external spermatic veins [9].

The variations of the venous anatomy may also be responsible for the varicocele recurrence [41]. A small study on 17 varicocele recurrences demonstrated that 11 (64.7%) of the patients exhibited Bähren type III anatomy (collaterals draining into a single gonadal vein with duplications being found most frequently in the pelvis and inguinal canal) (Table 27.1, Fig. 27.1) [42]. In some cases, ligation of the internal spermatic veins during the initial varicocele surgery may result in dilation of the external spermatic or gubernacular veins due to the increased flow of the venous blood. This phenomenon may also be responsible for recurrence in some varicocele patients [43].

Finally, the duration of follow-up may also have an impact on the varicocele recurrence. As the dilation of small-untied veins may take some time after the post-varicocelectomy alterations in the venous circulation, they may not be easily detected during physical examination in the early postoperative period. A retrospective chart

Table 27.1 Bähren et al. [42] classification of types of varicocele

Classification	Description
0	No reflux in gonadal vein
I	Reflux in single incompetent gonadal vein
II	Reflux to the main single gonadal vein tributing via multiple collaterals to lumbar or iliac veins, perivertebral venous plexus, or to inferior vena cava
III	Reflux to a duplicated gonadal vein where duplication can occur in the superior, inferior, or middle portion of the vein
IV	Reflux through renal hila or capsular veins when the renal-gonadal vein junction valve is competent
V	Reflux into a gonadal vein drained by additional (doubled) renal vein

Based on data from Ref. [42]

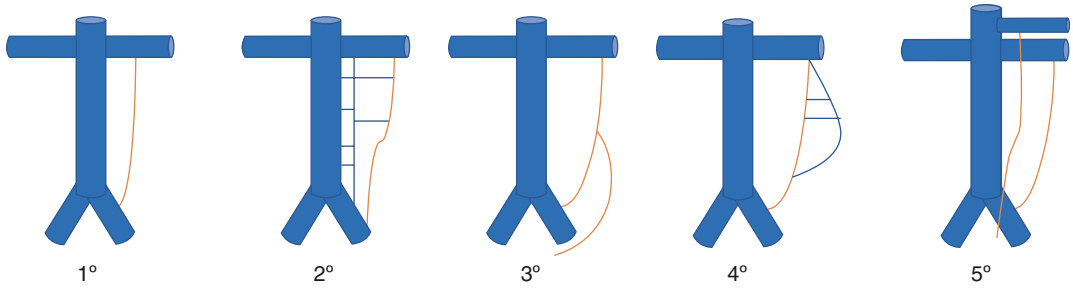

Fig. 27.1 Bähren et al. [42] classification of types of varicocele. (Based on data from Ref. [42])

review of adolescent varicocele patients demonstrated that recurrent varicoceles may be palpated even 76 months after the surgical repair where none had been detected at a mean of 27 months after varicocelectomy [44]. Another study investigated the association between the presence of persistent reflux flow on the first postoperative day and varicocele recurrence [38]. The authors examined the patients with ultrasonography for four consecutive times (preoperatively, 1st postoperative day, 3rd and 6th postoperative months). These findings revealed the need for establishing standards for the diagnosis of varicocele recurrence, and future studies are warranted for elucidating the clinical importance of diagnosing subclinical recurrent varicoceles.

Management of Recurrent Varicocele

There is scarce data on the optimal management of recurrent varicocele, and most of the studies are retrospective, with small sample size and heterogeneous population (Table 27.2). There are only four studies that assessed the efficacy of surgical treatment of recurrent varicoceles, whereas two studies reported the outcomes of embolization among these patients.

In their recent study, Yan et al. [45] reported their surgical experience with transumbilical single-port laparoscopic varicocelectomy (TUSPLV) on 64 patients with recurrent varicocele. The patients underwent either surgery using traditional retroperitoneal ligation of the internal spermatic vein (n = 30) or surgery using TUSPLV (n = 34). The results showed that the time of operation and bleeding volume in the TUSPLV group were significantly lower. Moreover, the complications were significantly lesser in the TUSPLV group compared to the patients who underwent traditional retroperitoneal ligation. In terms of the pregnancy rate, the difference between the two groups had no statistical significance. The authors concluded that employing TUSPLV to treat recurrent varicocele is safe and effective [45]. Although the feasibility of TUSPLV has been demonstrated in the primary varicocele cases [49], there are limited data on its efficacy and safety among patients with varicocele recurrence. More studies analyzing the cost-effectiveness of this surgery are needed before this treatment can be offered to recurrent varicocele cases.

Previous studies also demonstrated that subinguinal approaches may be as effective as high ligation techniques in the management of recurrent varicocele. Grober et al. [47] examined the outcomes after testicular artery and lymphatic-sparing subinguinal microsurgical varicocelectomy for varicocele recurrence on 54 men. The authors observed a significant increase in mean serum testosterone levels and mean testicular volume after the operation. Moreover, median sperm concentration and percent motility were also improved in the postoperative period, which was associated with an overall pregnancy rate of 40% in a mean 24-week follow-up period (via natural insemination, IUI, and IVF) [47]. In an older study, Madjar et al. [48] also reported the outcomes of 23 patients with clinical varicocele recurrence after high retroperitoneal or trans-

Table 27.2 The characteristics of the studies evaluating the efficacy of the surgical treatments of recurrent varicoceles

Authors	Design	Surgical technique	Outcome
Yan et al. (2017) [45]	Prospective	Transumbilical single-port laparoscopic varicocelectomy (TUSPLV) ($n = 34$) vs. traditional varicocelectomy ($n = 30$)	*Recurrence rate*: 6 (17.6%) (TUSPLV); 12 (40.0%) (varicocelectomy) *Pregnancy rate:* 20 (58.8%) (TUSPLV); 16 (53.3%) (varicocelectomy)
Chen et al. (2014) [46]	Retrospective	Subinguinal microsurgical varicocelectomy ($n = 48$)	*Predictive factors of success*: lower serum concentrations of FSH (<14.6 mIU ml^{-1}) and peak retrograde flow (<37.0 ml sec^{-1}), a longer time to recurrence (>10 months), a higher testicular volume (>27.3 cc) preoperatively, and a higher number of ligated veins (>6) during redo varicocelectomy
Grober et al. (2013) [47]	Retrospective	Subinguinal microsurgical varicocelectomy ($n = 1424$)	*Recurrence rate*: 0% *Pregnancy rate*: 40%
Madjar et al. (1998) [48]	Prospective	Subinguinal microsurgical varicocelectomy ($n = 23$)	*Recurrence rate*: no recurrence with 1-year follow-up *Improvement of semen parameters:* 19/23 (82.6%)

inguinal ligation [48]. All recurrent varicocele patients underwent macroscopic open varicocelectomy with subinguinal approach. After the resurgery, no recurrence within 1-year follow-up was observed, while 19/23 (82.6%) had marked improvement in semen parameters [48]. These findings reveal that whether a microscope was used or not, subinguinal varicocelectomy can be beneficial for the treatment of recurrent varicocele. Future controlled studies comparing the efficacy and safety of subinguinal approaches vs. high ligation techniques may reveal the optimal surgical technique for the treatment of recurrent varicocele.

Apart from surgical approaches, radiologic interventions are also gaining increasing interest for the treatment of varicoceles in the last decades. Endovascular embolization of varicoceles was first described by Laccariono in 1977, and various embolization techniques have been introduced since then [50]. The procedure is minimally invasive and it is commonly performed under local anesthesia, which is associated with less discomfort and more rapid recovery. The success rate of endovascular embolization of varicocele is reported to be around 92% [51]. On the other hand, a recent systematic review evaluated the outcomes of the varicocele embolization on 3505 patients and reported an average recurrence rate of 4.2% (11–0%, SD: 5.9) [51]. However, varicocele treatment with endovascular embolization is found to be the least cost-effective approach when compared to microsurgical and non-microsurgical varicocelectomy [52]; thus, it has been recommended to be used in recurrent varicocele cases.

There are only two studies which evaluated the outcomes of recurrent varicocele after embolization. Mazzoni et al. [53] reported the feasibility of antegrade sclerotherapy on 53 patients with no significant complications. Unsuccessful results occurred in only two out of the 55. However, the authors did not assess the fertility parameters of those patients [53]. In another study, Kim et al. [54] compared the embolization outcomes of 28 patients who had recurrent varicocele after laparoscopic, retroperitoneal, or inguinal ligation. Embolization was technically feasible in all but two cases (93%), and one patient was lost to follow-up. In the remaining 25 cases, 80% had complete resolution on physical examination, 16% had partial improvement, and 4% had no improvement at a median follow-up of 195 days. Although both studies showed promising outcomes for endovascular embolization, several factors (e.g., need for radiological equipment,

technical difficulties in finding the veins, cost of the procedure, exposure to the X-ray) limit its widespread use.

Considering the findings of the aforementioned studies, it is difficult to determine the optimal treatment option for patients with recurrent varicocele. More importantly, it is also challenging to identify which patients will benefit from the correction of their varicocele recurrence. In a retrospective study, Chen et al. [46] identified predictive factors of improvement in semen parameters after the repeat varicocelectomy in 21 infertile patients with recurrent varicocele. They reported that factors associated with successful redo varicocelectomy were lower follicle-stimulating hormone levels, lower peak retrograde flow on Doppler ultrasound, longer time to recurrence of varicocele, larger testicular volume preoperatively, and higher number of ligated veins during redo varicocelectomy [46]. More recent studies also confirmed the beneficial effects of redo varicocelectomies on sperm parameters, decreasing the need for the use of assisted reproductive techniques [55]. Therefore, clinicians should tailor their treatment choice according to the patients' characteristics.

Conclusion

Management of recurrent varicocele remains to be a clinical challenge. Although missing the spermatic veins during the initial varicocele repair is the most common etiological factor for the varicocele recurrence, anatomical variation may also be responsible for some cases. Therefore, correction of varicocele recurrence is indicated for patients who remain infertile or symptomatic after the initial surgery, whereas the best management for patients with recurrent varicocele is under debate. Surgical correction of varicocele recurrence can be performed with open varicocelectomy, laparoscopic varicocelectomy, or embolization; however, the lack of randomized clinical trials comparing the outcomes of these different techniques hampers our ability to recognize the most effective treatment option.

Review Criteria

An extensive search of studies examining the management of recurrent varicocele was performed using search databases such as PubMed, Scopus, Web of Science, and MEDLINE. The end date for these searches was March 2018. The overall strategy for study identification and data extraction was based on the following keywords: "varicocele," "recurrent varicocele," "management," "surgery," "varicocelectomy," "sclerotherapy," and "embolization." Articles that published languages other than English were also considered.

Data that were solely published in conference or meeting proceedings, websites, or books were not included.

Multiple Choice Questions and Answers

1. Grade 0 of varicocele according to Bähren classification is:
   (a) **No reflux in gonadal vein**
   (b) Reflux in single incompetent gonadal vein
   (c) Reflux to the main single gonadal vein tributing via multiple collaterals to lumbar or iliac veins, perivertebral venous plexus, or to inferior vena cava
   (d) Reflux to a duplicated gonadal vein where duplication can occur in the superior, inferior, or middle portion of the vein
2. Incidence of recurrent varicocele after open technique is:
   (a) 20%
   (b) **13.7%**
   (c) 30%
   (d) 40%
3. The higher recurrence rate seen with the open retroperitoneal or laparoscopic approaches is often attributed to:
   (a) The onset of a new varicocele
   (b) The reflux from the contralateral spermatic vein

(c) **The inability to ligate external gonadal (cremasteric) vessels or the external spermatic vein**
(d) None of the previous

4. Meta-analytic results demonstrated that recurrence after varicocele was lower:
   (a) **After microsurgery than after laparoscopic or open varicocelectomy**
   (b) After laparoscopy
   (c) After open varicocelectomy
   (d) No difference
5. Are there evidences about the management of recurrent varicocele?
   (a) **No randomized clinical trials have been yet published.**
   (b) There are few randomized clinical trials.
   (c) There are many randomized clinical trials.
   (d) There are some meta-analysis to this regard.

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Cost-Effectiveness Analysis of Varicocele Repair and Assisted Reproductive Technology

Darren J. Bryk and Sarah C. Vij

Key Points

- While in vitro fertilization and intracytoplasmic sperm injection are effective treatment options for an infertile couple, these procedures can be extremely costly and lead to financial and psychological strain on infertile couples.
- Performing varicocelectomy may be more cost-effective than initiating infertility management with intracytoplasmic sperm injection in many couples.
- Cost-effectiveness models in infertility are inherently flawed, so providers must counsel couples on the risks and benefits of all approaches.
- Varicocelectomy results in improved total motile sperm count for men with varicocele-associated oligozoospermia, allowing a couple to use less invasive and less costly form of assisted reproductive technology.
- Varicocelectomy in men with nonobstructive azoospermia and varicocele may spare some men from surgical sperm retrieval.

Introduction

Varicoceles are defined as an abnormal dilation or tortuosity of the veins within the pampiniform plexus [1]. Varicoceles are the most correctable known cause of male infertility [2, 3]. This abnormality is well-reported in the pediatric and adult male populations alike, with reported prevalence of 8–16% and 15%, respectively [4–6]; there is a much higher prevalence of varicoceles in men seeking treatment for infertility. Varicoceles are a well-known cause of impaired semen parameters, testicular hypotrophy, and, thus, subfertility in men. Despite multiple studies noting improvement in these abnormalities after varicocele repair [7–10], the role of varicocele repair in the era of assisted reproductive technology is debated. Further, given that several studies have shown no benefit in varicocelectomy with regard to pregnancy rates [11–13], it may be more cost-effective for a man with varicocele-associated infertility to proceed immediately to assisted reproductive technology (ART).

Surgical treatment of varicocele is recommended by the American Urological Association and the American Society for Reproductive Medicine for men with a palpable varicocele and abnormal semen parameters on semen analysis; if a man with a palpable varicocele is trying to conceive, the couple should have documented infertility, and the woman should have normal or correctable infertility prior to varicocele repair;

D. J. Bryk · S. C. Vij (✉)
Cleveland Clinic Foundation, Glickman Urological and Kidney Institute, Cleveland, OH, USA
e-mail: vijs@ccf.org

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for the pediatric population, surgical correction is recommended for adolescents with a varicocele and reduction in testicular size [14, 15]. Given that many fertility treatments are costly, the decision to correct a varicocele should take into account the financial implications for a couple if ART is or is likely to be an option for them in their future based on female factor.

The most commonly utilized surgical approach to varicocelectomy is the subinguinal microsurgical varicocele ligation in which all testicular veins within the spermatic cord are identified and ligated, with care taken to avoid the other important structures of the spermatic cord. The goal of varicocele repair in an infertile male is to improve semen parameters and, ultimately, pregnancy rates. Currently, pregnancy can be achieved via natural pregnancy or with the help of ART. In this chapter, we will review the relationship of varicocelectomy with ART. More specifically, we will examine the role of varicocelectomy in leading to the use of less invasive and cheaper assisted reproductive techniques, the outcomes of ICSI after varicocelectomy, and several studies that have determined costs of varicocelectomy compared to direct ART without varicocelectomy.

Varicocele Repair and ART

The goal of varicocele repair is to allow previously infertile or subfertile men and adolescents to improve their fertility potential. This is evidenced by increased growth of the affected testis (in adolescents), improved semen parameters or achieving pregnancy after varicocele repair. Often, couples are referred for ART due to impaired semen quality related to a varicocele in the male partner.

Cayan et al., in 2002, sought to examine the effect of varicocelectomy on the use of different assisted reproductive technologies [16]. In this study, the pre- and post-varicocelectomy semen analyses were compared in 540 men with documented infertility for 1 year or more and palpable varicocele. These men were divided into four groups based on their pre-operative total motile sperm count determined by the type of ART they would typically be recommended: 0–1.5 million motile sperm, 1.5–5 million motile sperm, 5–20 million motile sperm, and 20 million or greater motile sperm were candidates for ICSI, IVF, intrauterine insemination (IUI), and natural pregnancy, respectively. All patients had microsurgical varicocelectomy. Results from this study showed that 31% of the pre-operative ICSI candidates had improvement in semen quality that would allow them to use a less invasive ART. Fifty-three percent of pre-operative IVF candidates and 43% of IUI candidates would be candidates for IUI and natural pregnancy, respectively. Additionally, natural pregnancy was obtained in 16.5%, 31%, 38%, and 60% of pre-operative ICSI, IVF, IUI, and natural pregnancy candidates, respectively. Overall, pre-operatively, 28.6%, 14.6%, 27.9%, and 28.9% were candidates for ICSI, IVF, IUI, and natural pregnancy, respectively, based on total motile sperm counts; this can be compared to post-operative candidacy rates of 25.2%, 10.2%, 23.3%, and 41.3%, respectively. These findings are certainly meaningful as varicocele repair led to increased natural pregnancy rates and spared couples from ICSI, which is costly and associated with morbidity for the woman [17].

Similarly, Kamal et al. retrospectively reviewed 211 infertile men with varicoceles [18]. This study noted that total motile sperm count significantly improved from pre-operative to post-operative semen analysis overall in the entire group and when pre-operative sperm concentration is split into groups of less than and greater than 5 million sperm per mL. More recently, Samplaski et al. reviewed a prospectively collected database of 373 men with a palpable varicocele who underwent microsurgical subinguinal varicocelectomy [19]. This study divided the men into three groups based on pre-operative total motile sperm count: <5 million, 5–9 million, and >9 million corresponding to IVF, IUI, and natural pregnancy candidates, respectively. Post-operatively, all three groups had significant improvement in total motile sperm count. Additionally, similar to Cayan et al. [16], significant proportions from each group would be can-

didates for less invasive and cheaper modalities of ART based on their post-operative total motile sperm count.

Varicocele Repair: Are ICSI Outcomes Improved?

Despite the aforementioned data showing that varicocele repair can allow for a patient to use less invasive and less expensive ART or even for natural pregnancy, there is still a proportion of couples that will require ICSI after varicocele repair. Indeed, some men have improvement in only one or even no semen parameters after varicocele repair [20, 21]. Several studies, however, have shown improvements in sperm DNA fragmentation and decreased presence of reactive oxygen species after varicocele repair, which may improve pregnancy rates [22–26].

Several studies have examined the role of varicocele repair in combination with IVF/ICSI [27]. Esteves et al. performed a retrospective review of 242 men with clinical varicocele and infertility who underwent ICSI: 80 were treated with subinguinal microscopic varicocelectomy, and 162 were not treated prior to ICSI [22]. In the treated group compared to the untreated group, the rates of clinical pregnancies (60% vs. 45%) and live births (46.2% vs. 31.4%) were significantly different. In contrast to these findings, Pasqualotto et al. retrospectively studied 248 men who underwent ICSI, 79 men with an untreated grade 3 varicocele and 169 men with a history of grade 3 varicocele that were treated with microsurgical subinguinal varicocelectomy prior to ICSI [28]. This study found no difference in pregnancy rates between these two groups. Gokce et al. published the largest study to date evaluating the effect of varicocelectomy on ICSI outcomes [29]. Three hundred and six men with infertility and a clinical varicocele who underwent one cycle of ICSI were retrospectively reviewed. Of this group, 168 men underwent microsurgical subinguinal varicocelectomy, and 138 were spared surgery prior to ICSI. Similar to Esteves et al., this study noted significantly higher rates of viable pregnancy (62.5% vs. 47.1%) and live births (47.6% vs. 29.0%). It is notable that the study by Pasqualotto et al. included only grade 3 varicoceles, which could limit generalizability of that study's results, while Esteves et al. and Gokce et al. included all clinical grades of varicocele. A meta-analysis by Esteves et al. evaluated the benefit of varicocelectomy on ART outcomes [30]. This meta-analysis found an overall significant increase in the pregnancy and live birth rates, with no change in the miscarriage rate.

In addition to improving ICSI outcomes in oligozoospermic men, varicocelectomy may allow for return of sperm to the ejaculate in men with nonobstructive azoospermia [31, 32]. Ustuner et al. in a study of 19 men with nonobstructive azoospermia who underwent varicocele repair followed by microsurgical testicular sperm extraction (microTESE) noted significant improvement in testicular histology [33]. Inci et al. evaluated the role of varicocelectomy on ICSI outcomes in azoospermic men [34]. They reviewed a group of 96 men with history of varicocele and nonobstructive azoospermia who underwent successful microTESE. Of these 96 men, 66 had prior varicocele repair and 30 had varicocele present at time of sperm extraction. This study found no difference in the rate of clinical pregnancy, but did find that sperm retrieval rate was significantly higher. In contrast, however, Haydardedeoglu et al. compared 31 men with nonobstructive azoospermia with history of varicocele repair with 65 men with nonobstructive azoospermia who had not undergone varicocelectomy, all of whom underwent microTESE followed by ICSI. They noted statistically significant higher rates of sperm retrieval, clinical pregnancy, and live birth in the varicocelectomy group [35]. In a meta-analysis of varicocele repair in men with nonobstructive azoospermia, Esteves et al. reported that sperm retrieval was significantly higher in the varicocele repair group [36]. Pooled analysis of the studies by Inci et al. and Haydardedeoglu et al., however, showed that the odds for achieving clinical pregnancy and live birth were not significantly different between the treated and untreated groups (p-values 0.05 and 0.08, respectively). Conversely, Kirby et al. performed a meta-analysis of the same two stud-

ies and noted a statistically significant improvement in pregnancy rate in the azoospermic group that underwent varicocele repair (p-value 0.044) [37]. Odds for live birth rate, however, was not significantly different (p-value 0.052). Of note, there have been reports of natural pregnancy in patients with nonobstructive azoospermia after varicocele repair [38, 39], but neither Inci et al. nor Haydardedeoglu et al. reported such a finding [34, 35]. At present, management of varicoceles in men with nonobstructive azoospermia is controversial and requires appropriate counseling.

Cost-Effective Analysis: Varicocele Repair Versus Direct ART

Though ART has led to pregnancies in couples that would not have otherwise been able to conceive, it can be very expensive [40, 41]. Furthermore, most health insurance plans in the Unites States do not provide financial coverage for infertility treatments [42–44]. Varicocele repairs, on the other hand, are typically covered by insurance providers [45]. This is a unique issue in countries without universal healthcare, such as the United States. Only eight states (California, Connecticut, Massachusetts, Montana, New Jersey, New York, Ohio, and West Virginia) have laws mandating some form of insurance coverage for male factor infertility evaluation and treatment [43, 46]. Often, a couple desiring fertility treatment may have no or insufficient insurance coverage [47]. Out-of-pocket costs lead to significant financial and psychological strain on infertile men and their significant others. Additionally, couples with male factor infertility paid significantly more than couples with only a female factor [44, 47]. Wu et al. evaluated expenses of infertility care for 302 couples and noted that couples with male factor infertility paid significantly more (>$9000) than those with female factor infertility only.

In 1997, Schlegel examined cost-effectiveness of immediate ART versus varicocelectomy in men with varicocele-associated infertility [48]. This study compiled the results from 12 studies between 1974 and 1995 that compared a varicocelectomy group with a control non-varicocelectomy group. Estimated cost for varicocelectomy was $4019, while cost for ICSI cycle was $26,275, which included the estimated additional costs of surgical or maternal complications as well as risk of multiple gestations. In this study, the estimated total cost per live delivery of immediate ICSI was $89,091; this was corrected to $62,263 after sensitivity analysis, which utilized the best-reported pregnancy rates of an ICSI cycle. This is compared to the total cost per delivery after varicocelectomy, which was $26,268. Of note, the high cost of ICSI was largely attributed to the risks and costs of multiple gestations. Schlegel thus noted that it is more cost-effective to begin management of varicocele-associated male infertility with varicocele repair rather than starting with immediate ART.

Penson et al. examined the cost-effectiveness of four management strategies for infertile couples younger than 40 years old in whom varicocele was the presumed cause of infertility and abnormal semen analysis was present [49]. These management strategies were (1) observation, (2) varicocelectomy followed by IVF, if necessary, (3) gonadotropin superovulation plus IUI followed by IVF if IUI was unsuccessful, and (4) immediate IVF. Using multiple treatment strategies is a unique strength of this study. From the perspective of the healthcare payer (i.e., the insurance provider), the IUI/IVF approach was about as effective as the varicocelectomy/IVF approach, with 0.73 probability of live delivery compared to 0.72, respectively, but with an incremental cost per live birth of $561,000 compared to varicocelectomy/IVF. The IVF alone group was even more costly than the varicocelectomy/IVF and IUI/IVF strategies and slightly less effective with 0.61 probability of live delivery. From the patient perspective, however, varicocelectomy/IVF strategy cost more and delivered less than either observation or the IUI/IVF strategy. Penson et al. conclude that immediate IVF is not cost-effective when compared to the other two strategies. On sensitivity analysis, the threshold of live delivery rate after varicocelectomy for which ranking of treatment strategies changes is 22.3%; that is, when the delivery rate after varicocele repair is below 22.3%, the pre-

ferred strategy is IUI followed by IVF, if necessary, whereas the varicocelectomy/IVF strategy is more cost-effective if the live delivery rate is greater than 22.3%.

Navigating these decisions is difficult for providers and patients alike. Meng et al. created a decision model to clarify the initial management of varicocele-associated infertility [50]. The first decision for a male with varicocele-associated infertility is varicocelectomy or ART (ICSI for total motile sperm count less than 10 million or IUI for motile sperm count greater than 10 million, according to his model). The cost per pregnancy and pregnancy rate were analyzed. Overall, initial varicocele repair was more cost-effective than ART, except for men with pre-operative total motile sperm count ranging from 10 to 20 million, where IUI yielded lower cost per pregnancy than varicocelectomy ($9000 vs. $11,333). On subanalysis, in men with total motile sperm count less than 10 million, varicocelectomy was more cost-effective than ICSI when post-operative pregnancy rate was greater than 14%. In men with total motile sperm count greater than 10 million, varicocelectomy was more cost-effective than IUI only when post-operative pregnancy rate was greater than 45%. Thus, this study concludes that infertility management may be related to surgeon expertise and pregnancy outcomes after varicocelectomy repair.

A more recent study by Dubin et al. examined the cost-effectiveness of varicocelectomy in men with severe oligozoospermia, defined as total motile sperm count less than 2 million, who would otherwise undergo ICSI for infertility management. Most IUI studies have shown that a typical cut-off to predict a successful IUI outcome is total motile sperm count >5 million [45, 51]. This study sought to determine IUI outcomes in these patients after varicocelectomy. Approximately 10 out of 17 men in the study had total motile sperm count improve to greater than 2 million after varicocele repair. Of these ten men with improved motile sperm count, seven underwent IUI, two of whom achieved successful pregnancy; thus, cost per pregnancy in the IUI after varicocelectomy group is $35,924. Cost per pregnancy of IVF/ICSI without varicocelectomy was found to be $45,795. Lastly, cost per pregnancy of IVF/ICSI after varicocelectomy was $93,203. In this specific cohort of severely oligozoospermic men, varicocelectomy can potentially result in successful IUI, a cheaper and less invasive option compared to immediate IVF/ICSI. A specific weakness in this study compared to the prior cost-effectiveness studies is the very small sample size. Thus, this study may not lead to change in clinical practice, but could stimulate further hypothesis-driven research in this area.

A notable limitation to all of the aforementioned cost-analysis studies is that the calculations are based on multiple assumptions. Cost for procedures is extremely variable based on insurance plans, hospital systems and geography. For instance, Schlegel's estimated cost of IVF cycle assumes that the cost of the procedure was equivalent to the charges for said procedure [48]. Furthermore, the process of the infertility workup and evaluation for a couple is rarely standardized, but Schlegel included evaluation costs (office visits, medical testing, medications, etc.) based on an assumed timetable. Penson et al. notes that many assumptions were made in creating their cost-effectiveness models and reports that sensitivity analyses were performed at varying values over different ranges of clinical possibilities to assess whether the conclusions would change (there were no changes) [49]. Schlegel performs sensitivity analysis as well and notes no change in the overall conclusion. While Meng et al.'s analysis is still based on outcome probabilities taken from published sources, its strength is that it provides a decision model and further delineates such decisions based on degree of total motile sperm count [50]. Further costs that are difficult to accurately account for include work missed throughout the infertility evaluation and during recovery after surgery. One also cannot assign a cost to the emotional toll that a couple pays when waiting on improved sperm counts or function after varicocele repair or when waiting on the efficacy of the IVF/ICSI cycle. Dubin et al. notes that a full comprehensive cost analysis is likely too complex given that infertility is based on multiple male and female factors [45]. Still, the simplified approaches of the aforemen-

tioned studies provide strong evidence for the cost-effectiveness of varicocelectomy for oligospermic men compared to ART.

In men with nonobstructive azoospermia, the role of varicocele repair as it pertains to cost-effectiveness is less clear. Lee et al. performed a decision analysis of ART versus varicocelectomy in men with nonobstructive azoospermia. They concluded that microTESE was more cost-effective than varicocelectomy for the management of varicocele-associated nonobstructive azoospermia [52]. In sensitivity analysis, it was noted that if the rate of successful delivery after IVF/ICSI became less than 10% or varicocele repair led to a natural pregnancy rate greater than 40%, varicocelectomy would be more cost-effective. This, however, is quite unlikely, as reported rate of natural pregnancy in this population after varicocelectomy is approximately 1% [32, 53].

Conclusion

Overall, varicocele repair improves semen parameters in most men with varicocele-associated infertility. The ultimate goal is to improve pregnancy rates, which is less straightforward as couples may still require ART. After varicocelectomy, men may be able to achieve pregnancy with their partners naturally or with less invasive and less costly ART modalities. Thus, varicocele repair has an important role in infertility management as it reduces cost to the patient and the healthcare system [54, 55]. Additionally, varicocelectomy improves pregnancy and live birth rates in oligozoospermic men who subsequently require ICSI and also proves to be cost-effective compared to proceeding directly with ART. The effect of varicocelectomy prior to ICSI in nonobstructive azoospermic men has not yet been fully elucidated, but current data report that varicocelectomy is not cost-effective in this subset of patients compared to IVF/ICSI. Varicocelectomy is often covered by insurance companies in the United States, while ART is expensive and rarely paid for by insurance. While more prospective research is needed, the data to date indicate the net benefit of varicocele repair on male infertility.

Review Criteria

An extensive search of studies examining varicoceles, varicocelectomy, assisted reproductive technology, and the cost-effectiveness of varicocele repair compared to assisted reproductive technology was performed using search engines PubMed, Google Scholar, and OhioLINK. The studies included range from 1971 to 2018. Searches were performed mostly using the following keywords (individually or in combination): "varicocele," "varicocelectomy," "varicocele repair," "infertility," "male infertility," "assisted reproductive technology," "intracytoplasmic sperm injection," "cost," and "cost-effectiveness." Only articles published in or translated to English were included.

Multiple Choice Questions and Answers

1. Currently, what is the most commonly used technique for varicocele repair?
   (a) Open retroperitoneal varicocele ligation
   (b) Laparoscopic varicocele ligation
   (c) Non-surgical varicocele embolization
   (d) **Subinguinal microsurgical varicocele ligation**
2. What is the total motile sperm count cut-off most studies use to predict a successful intrauterine insemination outcome?
   (a) >2 million
   (b) **>5 million**
   (c) >1.5 million
   (d) >9 million
3. The effect of varicocelectomy on the use of assisted reproductive technology is that:
   (a) It can lead to the usage of a less invasive modality of assisted reproductive technology with good fertility outcomes.
   (b) It can result in minimal change in total motile sperm count necessitating intracytoplasmic sperm injection for a couple's best chance at fertility.
   (c) The rate of natural pregnancy does not change.
   (d) **a and b**

4. In men with varicocele-associated infertility, varicocelectomy followed by the assisted reproductive technology compared to assisted reproductive technology without varicocelectomy has:
   (a) **Increased pregnancy and birth rates with no difference in miscarriage rates**
   (b) Increased pregnancy and birth rates and decreased miscarriage rates
   (c) Increased pregnancy, birth, and miscarriage rates
   (d) Increased pregnancy rates with no difference in birth or miscarriage rates
5. In men with varicocele-associated oligozoospermia and infertility, the decision to proceed with varicocelectomy as infertility management:
   (a) Should always be considered first-line regardless of pre-operative total motile sperm count
   (b) Should never be considered first-line as it is not a cost-effective treatment
   (c) Should be considered first-line only in men with pre-operative total motile sperm count greater than 10 million; only then is it cost-effective
   (d) **Is cost-effective as a first-line treatment in men with pre-operative total motile sperm count less than 10 million compared to intracytoplasmic sperm injection if expected post-operative pregnancy rate is greater than 14%**

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Management of Pediatric and Adolescent Varicocele

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Alexander D. Doudt and Matthew S. Christman

Key Points

- Management of pediatric and adolescent varicoceles remains a challenging dilemma for the urologist and includes options ranging from active surveillance to open, microsurgical, or laparoscopic varicocelectomy to endovascular embolization.
- Evaluation should include physical exam, at a minimum, and may be augmented by scrotal ultrasound, hormonal evaluation, and/or semen analysis.
- Varicocele repair in adolescents should be considered when there is objective evidence *over time* of testicular size discrepancy, SA abnormality, and/or patient discomfort.
- Laparoscopic varicocelectomy may be the most utilized approach by pediatric urologists; however, microsurgical techniques are associated with the highest success rates and the lowest complication rates.
- In management of the youth varicocele, preservation of fertility is paramount; but additional studies are needed to determine the optimal management strategy with the best long-term paternity outcomes.

A. D. Doudt · M. S. Christman (✉)
Department of Urology, Naval Medical Center San Diego, San Diego, CA, USA
e-mail: matthew.s.christman3.mil@mail.mil

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Introduction

Despite a plethora of literature over the past ten years, the adolescent varicocele remains one of the most debated topics in pediatric urology. In adults, varicocele is the most common cause of male infertility, and indications for surgical intervention are well-established. Varicocelectomy has been shown to improve semen parameters and fertility. However, the vast majority of men with a varicocele have normal fertility. In contrast to adults, most adolescents present with an asymptomatic varicocele and unknown future fertility. Thus, there is considerable difficulty in determining who would benefit most from surgical intervention and who can safely be managed with continued observation.

Anatomy and Epidemiology

The arterial blood supply to the testicle consists of the testicular, vasal, and cremasteric arteries. There is free communication between the arteries at the level of the testicle, as well as the multiple venous sinuses that make up the pampiniform venous plexus. This plexus coalesces to form four venous outflow tracts – the testicular (internal spermatic), vasal, cremasteric, and the external pudendal veins [1]. The testicular vein is the dominant outflow tract, classically draining into the left renal vein on the left side and the inferior

vena cava (IVC) on the right side. Formation of a varicocele, an abnormal dilation of the pampiniform venous plexus, is predominantly the result of anatomic differences between the drainage patterns of the left and right testicular veins. Right angle insertion of the left testicular vein into the left renal vein, increased length of the left testicular vein, and relative decreased flow of the left renal vein in comparison to the IVC predispose to left-sided varicoceles by increasing the hydrostatic pressure within the left testicular vein [2].

In the pediatric population, pre-adolescent varicocele is rare. Prevalence increases with pubertal development to approximately 16% by the late teenage years, which is similar to the rate of the general adult population [3–5]. It is believed that puberty, specifically testicular enlargement and the concomitant increase in blood flow, plays a strong role in the development of a varicocele. Additionally, epidemiological studies have shown that adolescents with a varicocele tend to be heavier and taller but with a lower BMI [4, 6].

Evaluation

While a small minority of adolescent patients will present with a symptomatic varicocele, the vast majority will be referred for asymptomatic scrotal swelling. Thus, clinical evaluation focuses on the perceived risk factors for future infertility, which include varicocele grade, testicular volume, venous flow on ultrasound, hormonal evaluation, and semen analysis (SA).

Direct Measurement

Patients should be examined in a warm room in both the standing and supine positions. While the clinician is palpating the spermatic cord within the scrotum, the patient should perform a Valsalva maneuver. A positive test will generate a palpable distension of the scrotal veins, which represents transmission of increased abdominal pressure into the dilated pampiniform venous plexus. Grade I varicoceles are palpable only with a Valsalva maneuver. Grade II varicoceles are palpable with the patient in the standing position during normal breathing. Grade III varicoceles are visible with dilated and tortuous veins bulging through the scrotal skin. Subclinical varicoceles (Grade 0) are visible on ultrasound but not palpable with or without Valsalva.

When comparing varicocele grade to testicular size, the results have been mixed. Elder and Thomas retrospectively analyzed the records of 117 boys (mean age 13 years) with Grade II and III varicoceles. The incidence of testicular growth arrest, defined as at least 15% volume differential on caliper measurements, increased from 39% for Grade II varicoceles to 56% for Grade III varicoceles ($p < 0.01$) [7]. On the contrary, Alukal et al. retrospectively evaluated 168 boys (mean age 14.9 years old) and found no significant difference in mean testicular volume differential, as determined by ultrasound, with varicocele grade (18% for Grade I, 25% for Grade II, 19% Grade III, $p = 0.10$) [8]. While the influence of varicocele grade on semen quality in adult men is variable, no correlation has been observed in the adolescent population [9–11].

Due to the uncertain correlation of varicocele grade with semen quality, barriers to performing and interpreting semen analysis in the adolescent, and the fact that seminiferous tubules comprise 70–80% of testicular mass, testicular volume measurement has emerged as a surrogate for potential fertility. Total testicular volume (TTV) can be measured clinically with calipers or orchidometers. Both Prader orchidometry, which utilizes calibrated beads, and Rochester orchidometry, which utilizes punched-out rings, are popular methods. While there is a strong correlation between orchidometer and ultrasound for measurement of TTV, orchidometry consistently overestimates testicular volume and has a low sensitivity in detecting differences between left and right testicular volumes [12, 13].

Ultrasound

Ultrasound is the most accurate measurement of TTV and testicular asymmetry. However, it is

important to understand the limitations of this modality. These limitations include inter- and intra-observer variability, inter-institution variability, experience of the examiner, increased cost, differences in equipment, and differences in the properties of the transducer frequencies [14].

Testicular Volume

Scrotal ultrasound is best utilized as an accurate and reproducible measurement of TTV and testicular asymmetry [13]. Using the Lambert formula, volume = length × width × height × 0.71, individual testis volume can be calculated [15]. Ultrasound-derived TTV is associated with semen parameters in adolescents with a varicocele. In one study, a TTV <30 cc more than quadrupled the odds of a total motile sperm count (TMC) <20 million/cc [16].

Testicular Volume Differential

Testicular asymmetry can be calculated using two formulas – (1) testicular volume differential, TVDiff = (RTV − LTV)/TTV, and the (2) atrophy index, AI = (RTV − LTV)/RTV. RTV and LTV represent right and left testicular volumes. A simple conversion makes these two formulas interchangeable [17]. When comparing TVDiff and SA, TVDiff of 10–20% is associated with significantly lower sperm concentration and TMC. For TVDiff >20%, the association is more pronounced [11].

Peak Retrograde Flow

Despite the association of testicular asymmetry and abnormal semen parameters, a significant portion of adolescents can experience catch-up growth during a period of observation [18]. Peak retrograde flow (PRF) on duplex Doppler ultrasound has emerged as a predictor for persistent or progressive testicular asymmetry. PRF is performed by measuring the largest vein in the pampiniform venous plexus during Valsalva with the patient in the supine position. Kozakowski et al. found that 100% (14/14) of patients with both ≥20% testicular asymmetry and PRF >38 cm/sec on initial ultrasound had persistent asymmetry on follow-up ultrasound. The mean interval between ultrasound evaluations was 13.2 months [19]. In a follow-up study, 93% (41/44) of boys with ≥20% asymmetry and PRF >38 cm/sec had persistent asymmetry at a mean follow-up of 15.3 months. When factoring in boys with initial testicular asymmetry between 15% and 19.9% and PRF >38 cm/sec, 100% (9/9) had persistent asymmetry at a mean follow-up of 15.9 months. Catch-up growth was defined as <15% asymmetry [20]. Thus, many recommend that adolescent boys should not be followed conservatively when they present with testicular asymmetry >20% and PRF >38 cm/sec.

Endocrine Evaluation

The presence of a varicocele has been proposed to affect the hormonal markers of infertility and testosterone production. Multiple attempts have been made to identify early diagnostic markers of testicular dysfunction. Studies evaluating GnRH stimulation, inhibin B, and anti-Mullerian hormone have had modest but variable outcomes [21–24]. Practical concerns, such as cost of testing, multiple blood draws, and accounting for adolescents at varying Tanner stages, need to be factored into the equation. Currently, the role of hormonal evaluation in the diagnosis of the adolescent varicocele remains unclear.

Semen Analysis (SA)

SA is the most direct marker of fertility potential and is a vital component of varicocele evaluation in adults. In adolescents, it is well-known that a varicocele can have deleterious effects on multiple semen parameters [25]. Of those parameters, TMC has been shown to be the best predictor of fertility outcome [26]. While it is beneficial to have multiple SAs over a period of time to guide decision-making, even one SA can provide valuable information regarding potential future fertility [27]. However, ethical concerns and anxiety from urologists, patients, and parents about fertility-related issues and masturbation have limited the practice of obtaining a SA. In addition, there is significant difficulty in interpreting

SA, as there are no standard norms for adolescent semen parameters. Adult WHO standards are extrapolated to this group.

Management Options

In management of the youth varicocele, preservation of fertility is paramount. However, controversy exists in how best to accomplish this goal. In a 2014 study regarding practice patterns of pediatric urologists, 3% would operate on all varicoceles at time of diagnosis, 14% would observe, and 83% would gather more information before making a decision. The vast majority of respondents would operate for testicular asymmetry, but only 39% would intervene for altered semen parameters. In a patient with an asymptomatic varicocele and symmetric testes, 28% would operate based on varicocele grade alone [28]. There are inherent risks with both observation and surgical intervention. In order to maximize future fertility while preventing overtreatment, it is important for urologists to have a good understanding of management strategies and indications for repair.

Observation/Pre-intervention

Within a healthy adolescent population, approximately 15% of boys can have testicular asymmetry >20% [29]. During puberty, it is hypothesized that testes develop at different rates. Therefore, early surgical intervention may expose many boys to unnecessary risks.

Surveillance Strategies

As not all adolescents with a varicocele experience ipsilateral testis growth arrest or subfertility, active surveillance (AS) has emerged as a useful strategy. *In boys with an untreated varicocele, testicular catch-up growth occurs in 33–71% with expectant management alone* [18, 30–32]. Greater catch-up growth may occur with puberty progression (Tanner IV and V) [30]. Additionally, approximately one-half of Tanner V boys with an untreated varicocele, normal testicular volume, and initial TMC <20 million will experience normalization of TMC on follow-up SA [33]. These data suggest that there is a cohort of young men who can safely and reasonably be surveilled and, ultimately, spared surgery.

In contrast, non-operative management has been associated with persistent testicular asymmetry. In one retrospective study, 181 patients were followed expectantly with serial ultrasound (79%) or orchidometer (21%) measurements. After a median follow-up of 12 months, 35% (37/105) had progression of testicular asymmetry to >20%, while 53% (35/66) had persistent asymmetry >20% [32].

Multiple active surveillance algorithms have been proposed, but no standard protocol exists. *Regardless of algorithm, emphasis is on consistent testicular volume measurement for at least one year*. Ultrasound is superior to orchidometry and should be utilized as the imaging modality of choice. However, cost considerations may lead to favoring orchidometry over ultrasound. Orchidometry has a strong correlation to ultrasound and can also be utilized as a valid monitor of testicular growth over time [34]. One author proposed annual follow-up for boys with TVDiff >20% and biennial follow-up for those with TVDiff <20% [35]. Discussion between the patient and family regarding the role for SA should be performed when the patient reaches Tanner V. If there is evidence of subfertility (TMC <20 million), then serial SA is recommended (if surgery is not elected at that point).

Indications for Repair

Varicocele repair in adolescents should be considered when there is objective evidence of testicular size discrepancy, SA abnormality, or patient discomfort. There is no consensus on the threshold for testicular asymmetry, but greater than >20% or >2 mL has been utilized by many institutions. Care should be taken when utilizing strict volume differences, as a 2 mL difference in a Tanner Stage I or II boy is vastly different than in a Tanner Stage V boy. *The decision to proceed with surgical intervention should be based on multiple measurements*

performed in a consistent manner and obtained over a period of time. There is no current role for repair based on varicocele grade, testicular consistency, or the presence of a subclinical varicocele. Although, observation for a subclinical varicocele may be warranted as up to 28% may progress to a clinically detectable varicocele [36].

Intervention

While varicocelectomy in the adolescent boy is a low morbidity surgery, it is not without complications. The risks and benefits need to be thoroughly discussed prior to the decision to proceed with treatment (Table 29.1). Hydrocele formation, due to lymphatic disruption, and varicocele recurrence are the two most common complications. Testis atrophy and nerve injury have also been reported but are exceedingly rare. There are multiple surgical approaches to varicocele. All involve ligation of the engorged spermatic veins. Differences are based on access to the testicular vessels, level of ligation, and whether the testicular artery and lymphatic vessels are spared or ligated.

Open

High ligation of the spermatic vessels, en masse, is known as the classic Palomo technique. High retroperitoneal exposure allows for two distinct advantages over the inguinal or subinguinal approach – (1) dissection is superior to the vas deferens, and (2) there is often only a single internal spermatic vein to ligate. A short, transverse incision is made two fingerbreadths medial to the anterosuperior iliac spine. The retroperitoneum is entered using a muscle-splitting incision. The peritoneum is retracted medially, exposing the testicular artery and internal spermatic vein on the posterior aspect of the peritoneum. The vessels are isolated with a vessel loop and elevated into the operative field. Bulk ligation of the spermatic cord is performed using a 3-0 silk suture. At this level, the vas deferens and the deferential artery are well inferior. In obese adolescents, exposure can be difficult. Traction on the ipsilateral testis can aid in identifying the cord structures. A modified Palomo technique can be performed by preserving the testicular artery.

In 2008, Feber and Kass reported their outcomes with the classic open Palomo technique in 233 adolescent boys (mean age 14.6 years).

Table 29.1 Management options

Surgical approach	Hydrocele rate	Failure rate	Testicular catch-up growth	Advantages	Disadvantages
Open					
Retroperitoneal (Palomo) [38]	9.7%	2.9%	37–100%	Dissection superior to vas deferens	Reported hydrocele rate as high as 29% [37]
Inguinal (Ivanissevich) [39]	10.0%	15.0%	70% [69]	Easy access to spermatic cord	High recurrence rate
Microsurgical					
Inguinal [42, 70]	0–6.4%	0–4.2%	75%	Improved visualization of peri-arterial and collateral veins	Need for microsurgical training. Increased venous branching (subinguinal) → increased operative time
Subinguinal [41]	1.0%	1.0%	70–85%		
Laparoscopic					
Laparoscopic transperitoneal [38]	6.9%	4.4%	37–100%	Technical ease, short operative time	Potential injury to abdominal organs/vessels
Endovascular					
Retrograde embolization [54]	0%	13.0%	—	Lymphatic-sparing, artery-preserving technique	High recurrence/persistence rates, radiation exposure
Antegrade embolization [55]	0–1.0%	12.0%	93%		

Persistent varicocele was noted in 3.9% (9/233) of patients, while secondary hydrocele was reported in 29% (68/233) [37]. In a 2009 systematic review, the rate of hydrocele formation following the classic open Palomo technique was 9.7%. When comparing the classic versus modified techniques, hydrocele formation was significantly lower in the modified group (7.7% vs. 3.2%, $p < 0.001$). There was no difference in recurrence rates between the two groups (3.4% vs. 4.2%, $p = 0.506$) [38]. Of note, the authors did not differentiate laparoscopic versus open techniques during comparison.

Open inguinal repair (Ivanissevich) is performed by making a small groin incision just superior to the lateral aspect of the ipsilateral pubic tubercle. The external oblique aponeurosis is incised along the direction of its fibers. Care is taken to avoid the ilioinguinal nerve. The spermatic cord is isolated with a vessel loop or Penrose drain. Under loupe magnification, the engorged internal spermatic veins are clipped or suture ligated with a fine silk suture. The vas deferens and testicular artery are preserved, although some surgeons prefer ligating the testicular artery. Reverse Trendelenburg position can be utilized to identify any remaining engorged veins. A subinguinal repair is performed in a similar manner to the inguinal approach, except the incision is made directly over or below the external inguinal ring. Without the use of an operating microscope, inguinal varicocelectomy historically has a reported recurrence rate of 15% with a secondary hydrocele formation rate of 10% [39].

Microsurgical

The use of the operating microscope for the inguinal and subinguinal approaches allows increased magnification (6× to 25×) to more accurately identify small remaining peri-arterial and collateral veins, as well as preserve arteries, lymphatics, and nerves [40]. The results have mirrored those in the adult literature with exceedingly low rates of hydrocele formation and varicocele recurrence rates less than 3% [41, 42]. Delivery of the ipsilateral testis may reduce rates of recurrence to practically zero [42]. Despite these advantages, between 2003 and 2012, only 2% of adolescent varicoceles were performed using the microsurgical approach [43]. It is hypothesized that lack of familiarity and inexperience with advanced microscopic techniques among pediatric urologists may play a significant role in this trend.

Laparoscopic

Laparoscopic varicocelectomy has emerged as a minimally invasive alternative to open and microscopic surgery. Advantages include rapid patient recovery, minimal morbidity, technical ease, short operative time, and magnified visualization well above the vas deferens and deferential artery. Additionally, in the event of bilateral varicoceles, repair can be performed without the need for a second incision. Disadvantages include increased cost, potential injury to abdominal organs or vessels, and inherent risks of general anesthesia and pneumoperitoneum.

For a transperitoneal approach, pneumoperitoneum is established at the umbilicus using a Veress needle or open Hasson technique. A 5 mm or 10 mm umbilical camera port is placed. Pneumoperitoneum is maintained at 12 cm H_2O. Two additional 5 mm working ports are placed under direct visualization, so as to triangulate toward the internal ring on the pathologic side. Alternatively, a 5 mm and 3 mm working port may be utilized. The patient is placed in steep reverse Trendelenburg and rotated slightly away from the pathologic side so as to facilitate retraction of the bowels. The posterior peritoneum is grasped 4–5 cm proximal to the internal ring and slightly lateral to the spermatic vessels. A short "T-shaped" incision is made in the posterior peritoneum to expose the vessels. The spermatic veins are mobilized, elevated, clipped with a 5 mm vascular clip applier, and divided. Some surgeons advocate clipping and dividing both the artery and the vein. Fascial closure is performed for the 5 mm and 10 mm ports only.

Despite rates of hydrocele formation that are similar to the open Palomo technique, laparoscopic varicocelectomy has been widely adopted by pediatric urologists [38, 44, 45]. Approximately one-half of all pediatric varicocelectomies are performed laparoscopically [43, 46]. In one large-scale retrospective analy-

sis of academic pediatric urology centers, the rate of hydrocele formation following laparoscopic varicocelectomy was 8.1% (43/530) [46]. However, reported rates have been as high as 30% [44]. As a result, lymphatic-sparing techniques have been adopted. Multiple studies have reported on the use of preoperative intra-scrotal (sub-dartos, intravaginal, or intratesticular) injection of dyes in order to stain the lymphatic vessels for easier intra-operative identification. In one retrospective single institution study, the rate of hydrocele formation decreased from 10.8% (25/230) to 0% (0/105) following the adoption of a lymphatic-sparing technique with isosulfan blue [47]. However, concerns still exist regarding the potential for dye-induced testicular damage and residual pigmentation of the scrotum, which can persist for up to 3 months [48, 49]. Other lymphatic-sparing techniques have shown similar efficacy rates. Glassberg and colleagues reported a significant decrease in the incidence of post-operative hydroceles (3.4% vs. 11.4%, $p = 0.025$) after adding laparoscopic cord skeletonization to their technique [50].

Controversy exists regarding the safety of testicular artery ligation. The majority of surgeons tends to preserve the testicular artery due to the potential risk of testicular atrophy and worsened infertility. However, critics cite collateral blood flow to the testis and the increased risk of persistent or recurrent varicocele as a reason to ligate the spermatic cord en masse. In 2016, Yu et al. retrospectively evaluated 122 patients (mean age 17.3 years) who underwent laparoscopic varicocelectomy with artery preservation ($n = 57$) or artery ligation ($n = 65$). There was no difference in the rate of varicocele recurrence between the two groups (5.3% vs. 3.1%, $p = 0.881$) [51]. Regardless of technique, varicocele recurrence or persistence rates following laparoscopic varicocelectomy are consistently less than 8% [47, 50, 52].

Endovascular

Endovascular embolization is a minimally invasive alternative to surgical intervention with the potential advantages of no general anesthesia, no incision, and low morbidity. It is considered a lymphatic-sparing and testicular-artery-preserving technique. However, historically low success rates, high recurrence rates, radiation exposure, and potential need for an experienced interventional radiologist have limited its utility in the first-line setting. One single institutional study retrospectively evaluated 40 boys (mean age 13.1 years) undergoing retrograde embolization. After a single procedure, overall success rate was 60% (24/40) with a 27.5% (11/40) technical failure rate and a 10% (4/10) recurrence rate [53]. Recently reported success rates for retrograde embolization have been more acceptable, but recurrence rates remain high [54]. Antegrade embolization has shown more promise [55, 56]. The procedure is performed under general or local anesthesia. A small subinguinal or high scrotal incision is made, and the spermatic cord is isolated with a vessel loop. The most dilated vein is isolated. After obtaining proximal and distal control, the vein is cannulated and venography is performed. Care is taken to avoid radiation exposure to the scrotum. During injection of the sclerosing agent, pressure is applied to the ipsilateral hypochondrium, or if the patient is awake, he is instructed to Valsalva. Once complete embolization is documented on fluoroscopy, the cannula is removed and the vein is suture ligated. Overall, additional studies are needed to better clarify the role of endovascular techniques in the first-line setting.

Post-intervention

Measuring Success

A strict definition of success following varicocelectomy remains elusive. Outcomes measured include resolution of pain, varicocele cure on physical examination or ultrasound, testicular catch-up growth on ultrasound or orchidometry, assessment of endocrine and semen parameters, and paternity. Due to the significant heterogeneity in primary endpoints within the literature, it has been difficult to compare the various surgical approaches.

Semen Analysis (SA)

While paternity is the ultimate patient goal, SA is critical to predicting future fertility potential. Multiple studies have reported improvement in semen parameters following varicocelectomy. In one retrospective study, 17 Tanner V adolescents with a clinical left varicocele and no testicular asymmetry underwent pre- and post-operative semen analyses. Median age at time of surgery was 18.2 years. Following varicocelectomy, median TMC significantly improved from 2.8 million to 18.2 million ($p < 0.01$). Primary benefit was observed in the abnormal preoperative TMC group, of which 82% had improvement in TMC and 55% had normalization [57]. In a prospective study involving 100 adolescents (mean age 14.7 years) who underwent subinguinal or inguinal varicocelectomy with varying degrees of magnification, there was a statistically significant improvement in TMC (22.6 million to 64.53 million, $p = 0.002$) following surgery [58]. In a 2014 meta-analysis involving 10 prospective trials, youth varicocelectomy resulted in a significant improvement in sperm density (+14.6 million/mL, $p < 0.001$) and motility (+6.6%, $p = 0.004$) [25]. While it is still unknown if the adolescent varicocele is the same as the adult varicocele, the beneficial effect of varicocelectomy on semen parameters appears to be similar.

Testicular Volume

Testicular asymmetry is commonly used as a surrogate for testicular dysfunction and spermatogenic potential. However, there is no consensus on what constitutes asymmetry. In a recent survey of Society for Pediatric Urology members, 58.7% of respondents defined asymmetry using the >20% volume cutoff. The remaining respondents focused on increasing discrepancy over time (31.1%) and >10% volume cutoff (6.6%). Size discrepancy was not utilized in 3.6% [59]. These discrepancies have made it difficult to compare testicular volume changes between studies. Nonetheless, these studies have been performed and have provided valuable insight into catch-up growth rates after surgery.

In a 2012 meta-analysis involving 14 studies and 1475 adolescents, the effects of surgical intervention on catch-up testicular growth were examined. Asymmetry was defined as (1) ≥10% or (2) ≥20%. Combined analysis showed that testicular volume discrepancy significantly decreased after surgery in both groups. In the 10% group, catch-up growth was seen in 73.7% of patients. In the 20% group, 77.9% of patients experienced catch-up growth [60]. Additionally, Spinelli and colleagues prospectively analyzed 54 consecutive youths (median age: 14.5 years) who had a left varicocele and testicular asymmetry >20%. Half of the cohort underwent lymphatic-sparing microsurgical varicocelectomy, while the remaining half underwent AS. After 12 months, 85.2% of the intervention group experienced testicular catch-up growth [42]. Within the control group, 29.6% experienced catch-up growth, which is consistent with previously published reports of asynchronous testis growth [32]. Whether the significant increase in testicular volume following varicocelectomy is truly an increase in seminiferous tubules or a function of testicular edema remains to be seen [61].

Hormone Production

In adults, varicocelectomy has been shown to have a positive impact on testosterone levels [62]. In adolescents, few studies have examined low testosterone as an early marker for infertility or the effect of varicocelectomy on testosterone levels. One recent observational, single institution study involving 408 adolescent boys found that microscopic inguinal or subinguinal varicocelectomy significantly increased total testosterone levels compared to conservative management [63]. Studies involving other endocrine factors, such as GnRH stimulation, LH response, FSH, and inhibin levels, have shown mixed results. Further research is needed to clarify the effect adolescent varicocele has on the hypothalamic-pituitary-gonadal axis before endocrine factors can be considered as a meaningful measurement of success following surgery.

Paternity

Paternity is the definitive marker of male fertility. However, it is difficult to evaluate as female factors, socioeconomic status, and cultural or per-

sonal beliefs all play an influential role in one's ability and decision to conceive. Additionally, well-done studies that use paternity as a primary endpoint would require significant time and resources. Initial studies reported paternity rates of 100% (18/18) and 75% (12/16) following adolescent varicocelectomy [64, 65]. However, these studies were limited by the lack of a control arm. In 2013, Bogaert et al. surveyed 661 men, of which 372 previously underwent antegrade sclerotherapy (mean age 15.3 years) and 289 were followed conservatively (mean age 17.1 years). After factoring in rates of response (53%) and desire to have children (45%), there was no significant difference in paternity between the conservative group (85%, 61/72) and the varicocelectomy group (78%, 67/86) [66]. As endovascular techniques have lower rates of success and high rates of recurrence when used in the first-line setting, the findings of this study are limited due to the sole use of antegrade sclerotherapy in the treatment group. Most recently, Cayan and colleagues compared 286 adolescents who underwent microsurgical subinguinal or inguinal varicocelectomy and 122 adolescents who elected against surgery and thus were followed conservatively. In this single institution observational study, paternity rates were 77.3% (222/286) in the treatment arm and 48.4% (59/122) in the control arm ($p < 0.005$). Mean time to conception was significantly shorter in the surgery group (11.18 months vs. 16.85 months, $p < 0.005$) [63]. While these findings provide direct evidence of the positive impact early varicocelectomy has on the fertility of adolescents, additional studies are needed to determine if these preliminary findings are generalizable.

Treatment of Recurrent Varicocele

Recurrence rates following varicocelectomy range from 0% to 35% and depend on the type of approach and the use of magnification. As the testicular blood supply may be compromised from the initial surgery, the need for redo surgery can result in significant anxiety for the patient, parents, and the surgeon. Indications for repair are based on the same factors that prompted the initial surgery. Endovascular techniques are often recommended as the approach of choice, as they pose the least risk to the blood supply of the testis. If a surgical approach is chosen, consideration should be given to the initial repair so as to operate in a virgin field. In one study, open redo varicocelectomy was successfully performed in 17 out of 17 boys. However, one patient developed testicular atrophy, and three patients developed a hydrocele that required surgical repair [67].

Conundrums in Varicocele Management

Adolescent Varicocele in the Absence of Pain: Observe or Operate?

While the last decade has provided a plethora of new knowledge regarding the asymptomatic adolescent varicocele, questions still remain regarding the future impact on fertility, the best diagnostic marker for early testicular dysfunction, and how to define success following surgical intervention. Currently, there is no consensus on when to operate, but the decision to proceed with surgery should focus on objective evidence of testicular size discrepancy and/or SA abnormalities documented in a consistent manner over time. There is hope that the next decade of research will fill in these gaps and provide pediatric urologists with the tools to offer a concise, evidence-based answer to patients and parents when asked, "should we observe or operate?"

Pre-adolescent Varicocele: What Does this Really Represent?

The adolescent varicocele is believed to be related to the increase in testicular blood flow mediated by puberty. When a pre-adolescent develops a varicocele, it presents a unique situation to the pediatric urologist. Is the pre-adolescent varicocele simply a varicocele in a boy who has initiated puberty at a younger age? Or is this a wolf in sheep's clothing – a

paradigm shift that changes our decision-making and management? Evaluation in this age group is limited to monitoring for testicular asymmetry and potentially assessing PRF. Asynchronous testis growth can be more pronounced during the early Tanner stages of pre-adolescence, which can confound the situation. Unless boys are more physically developed, there is no role for semen analysis. Even if they are able to masturbate, significant ethical considerations and challenges in defining normal semen parameters remain. There is a void in the literature regarding this topic, which makes it ripe for future studies and hopefully some answers to these questions.

Ethics of Semen Analysis in the Adolescent Population: When to Ask for SA?

Pediatric urologists often face apprehension when confronted with the idea of asking an adolescent boy to provide a SA. There is a perception that patients are too young to be asked to provide a semen sample or to be labeled as subfertile. In a 2016 survey of pediatric urologists, 53% never asked for a SA, 13.1% routinely asked for SA, and 23.8% asked for SA if their appeared to be interest from the patient or family. Among those who utilized SA, 45% used a cutoff of age greater than 18 years, 21% used greater than 17 years, and 17% used Tanner V [59]. There is no strict age cutoff for obtaining a SA, but what defines normal semen parameters for the adolescent is unknown. Reference ranges are extrapolated from the adult WHO criteria. Therefore, boys should at least be Tanner V before being approached with the request for a semen sample. Patients and parents often times feel uncomfortable when presented with the idea of providing a SA. There is embarrassment in discussing sex-related issues and a lack of knowledge regarding SA and its collection methods. Research has shown that early education on the part of the physician can bridge the gap in knowledge, improve patient/parent comfort level, and increase satisfaction in the process [59, 68].

Conclusion

In conclusion, the decision to proceed with surgical intervention should focus on objective evidence of testicular asymmetry, abnormalities in semen parameters, or patient discomfort. As a significant portion of adolescents may experience spontaneous testicular catch-up growth, active surveillance has emerged as an effective strategy. Laparoscopy is the approach of choice among many pediatric urologists, but microsurgical inguinal or subinguinal approaches have the highest success rates with the lowest complication rates.

Review Criteria

A thorough review of studies on the topic of adolescents and varicocele was conducted. The PubMed, Embase, Medline, and Web of Science databases were utilized with keywords including "varicocele," "varicocelectomy," "youth," "adolescent," and "pediatric." There were no limits on dates of publication. The review was limited to articles published in the English language, and only peer-reviewed and published literature was evaluated. No data were considered otherwise.

Multiple Choice Questions and Answers

1. Which of the following treatment options for varicoceles has the highest success rate and the lowest complication rates?
   (a) Open Palomo technique
   (b) Laparoscopic Palomo technique
   (c) Endovascular embolization of the gonadal vein
   (d) **Microsurgical varicocelectomy**
2. A 16-year-old boy is referred to the pediatric urology clinic for asymptomatic scrotal swelling. On exam, he is noted to have a Grade III left varicocele. Hormonal evaluation reveals a testosterone of 350 ng/dL and normal LH/

FSH. Scrotal ultrasound is obtained and reveals left testicular volume is 21% less than the right. The patient is placed on active surveillance, and 1 year later, he undergoes a repeat ultrasound which reveals testicular asymmetry has worsened to 25%. A semen analysis shows total motile sperm count (TMC) of 15 million per ejaculate. What is not an indication for surgical intervention?
   (a) TMC 15 million per ejaculate
   (b) Testicular asymmetry 25%
   (c) **Grade III left varicocele**
   (d) Testicular asymmetry 21%
3. A 17-year-old young man underwent an inguinal microsurgical left varicocelectomy. At 1 year follow-up, he is noted to have a recurrent Grade II left varicocele with testicular asymmetry of 23% on ultrasound. Semen analysis reveals a total motile sperm count of 12 million per ejaculate. What is the next best step in management?
   (a) Repeat microsurgical varicocelectomy
   (b) Repeat semen analysis in 6 months
   (c) Repeat scrotal ultrasound in 6 months
   (d) **Antegrade embolization**
4. Barriers to the utilization of semen analysis (SA) in the evaluation of adolescents with a varicocele include all of the following, except:
   (a) Lack of standardized normal values of SA in this population
   (b) **Difficulty finding a lab that will analyze an adolescent's SA**
   (c) Embarrassment of the physician, adolescent, or parent
   (d) Stigma associated with diagnosing an adolescent with low sperm counts
5. A 13-year-old Tanner Stage III boy is referred to the pediatric urology clinic for asymptomatic scrotal swelling. On exam, he is noted to have a Grade II left varicocele. Prader orchidometry reveals the left testicular volume is 25% less than the right. Scrotal ultrasound is performed and reveals testicular asymmetry of 20% with a peak retrograde flow of 20 cm/sec. What is the next best step in management?
   (a) Semen analysis
   (b) **Active surveillance with repeat scrotal ultrasound in 1 year**
   (c) Active surveillance with repeat orchidometer measurement in 1 year
   (d) No further follow-up warranted
6. Approximately what percent of adolescents will be found to have a varicocele?
   (a) 5%
   (b) **15%**
   (c) 30%
   (d) 50%
7. All of the following ultrasound measurements/findings have been utilized in the management of pediatric and adolescent varicoceles, except:
   (a) Total testicular volume
   (b) Peak retrograde flow
   (c) **Testicular microlithiasis**
   (d) Testicular volume differential

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Part IV

Controversies Surrounding Varicocele

Is Varicocele a Bilateral Disease?

30

Peter Ka-Fung Chiu and Chak-Lam Cho

Key Points

- Unilateral clinical varicocele can affect both testes, leading to overall decline in fertility.
- In men with clinical left varicocele, there is a high prevalence of concomitant right varicocele on imaging studies. Under-diagnosis and under-treatment of low-grade right varicocele may contribute to the limited effect of left varicocele treatment in some men with infertility.
- There is no direct correlation between varicocele grade and testicular dysfunction.
- Bilateral varicocelectomy appears superior in improving semen parameters and pregnancy rate compared to unilateral repair in patients with clinical varicocele on one side and contralateral subclinical/lowgrade varicocele.

Introduction

Varicocele is classically described as a predominantly left-sided disease, with 85–90% cases showing unilateral left varicoceles, 0.4% unilateral right varicoceles, and 10% bilateral varicoceles [1].

The reason of its left-sided predominance is not well explained but is postulated to be related to incompetent valves in the left internal spermatic vein, right angle insertion of the left internal spermatic vein to the left renal vein, higher venous pressure in left internal spermatic vein, and "nut-cracker" phenomenon (left renal vein compression between the aorta and the superior mesenteric artery) [2].

The management of varicocele classically focuses on unilateral clinical varicocele in the majority of patients. This is particularly well illustrated in the management of adolescent varicocele that ipsilateral testicular hypotrophy is considered as an indication for intervention [3]. Indeed, a single normal testis is generally sufficient for normal fertility as observed in patients with unilateral undescended atrophic testicle and patients with orchidectomy performed for other reasons [4]. Therefore, the current belief is insufficient in explaining the overall decline in fertility in patients with unilateral clinical varicocele. It is a logical deduction that a clinical left-sided varicocele has to exert its deleterious effect on the contralateral testis to result in poor semen quality and subsequent subfertility via certain mechanisms. An alternative or co-existing reason would be under-diagnosis or

P. K.-F. Chiu
Division of Urology, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

C.-L. Cho (✉)
S.H. Ho Urology Centre, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

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under-grading of right-sided varicocele, and it is possible that a small or even subclinical right varicocele might add on the detrimental effect of a high-grade left varicocele. More recent studies have reported a much higher prevalence of bilateral varicoceles when colour Doppler ultrasound (CDUS) or venogram was used for diagnosis, but the exact prevalence is highly variable due to different study designs and diagnostic modalities [5, 6].

The implication of subclinical right varicocele is not yet fully elucidated, and whether a subclinical varicocele would progress to a clinical varicocele is a concern and a matter of debate. However, there is preliminary data to suggest that a subclinical or low-grade varicocele should not be left untreated particularly in the presence of a high-grade varicocele over the other side [7]. The suboptimal management may result in a less than satisfactory surgical outcome and mask the true beneficial effect of varicocele repair. Nonetheless, the additional benefit of bilateral over unilateral varicocelectomy on semen parameters and fertility in patients with clinical varicocele on one side and contralateral subclinical/low-grade varicocele requires further validation from well-designed randomized controlled studies.

In this chapter, the effect of unilateral varicocele on both testes is first illustrated. Then, a high prevalence and possible under-diagnosis of bilateral varicocele are discussed. It is followed by debate on the implication of subclinical/low-grade varicocele. Lastly, data suggesting the superior outcome of managing varicocele as a bilateral disease are listed.

Effect of Unilateral Left Varicocele on Both Testes

Scrotal temperature is kept lower than body temperature to facilitate spermatogenesis, and it is believed that a countercurrent heat exchange system helps to maintain a lower scrotal temperature. The presence of varicoceles may reduce the effectiveness of this heat exchange system and result in increased scrotal temperatures and subsequently impaired spermatogenesis [2]. Goldstein and Eid have shown that in men with unilateral left varicoceles, bilateral scrotal surface temperatures and bilateral intratesticular temperatures were found to be elevated compared with controls [4]. Animal studies with artificially induced left varicocele have shown reduction of serum testosterone and testicular degeneration on microscopy in the right testis in the presence of clinical left varicocele alone, in association with increase in bilateral intratesticular temperature [2, 3].

Microscopic changes on testicular biopsy were also observed in humans. More importantly, severity or grading of varicocele does not correlate well with semen quality and histopathologic patterns in testicular biopsy. Dubin and Amelar reported in 1970 that the size of varicocele does not predict the improvement of semen quality. Eighty-two percent of small-sized varicoceles, 81% of moderate-sized varicoceles, and 78% of large-sized varicoceles in 111 patients had improvement of semen quality after varicocele ligation [8]. Dubin and Hotchkiss also reported, in testicular biopsy of infertile men with left-sided varicoceles, structural abnormalities in tubules and sperm were observed in both testes, and the degree of histological abnormalities did not correlate with severity of varicocele [9]. Saleh et al. reported, in 37 azoospermic men with clinical bilateral varicoceles, the degree of abnormal histology shown in testicular biopsies did not differ significantly between grade 1, 2, and 3 varicoceles [10]. Therefore, a lower-grade clinical varicocele does not mean the varicocele affect the testicular function to a lesser degree.

Current evidence supports that unilateral varicocele can affect the contralateral side by raising scrotal or testicular temperature, and similar pattern of impairment in tubular structure and spermatogenesis were observed over both testes. A low-grade varicocele, which may go unnoticed or left untreated, might affect testicular function to a similar degree as high-grade varicoceles.

Prevalence of Bilateral Varicocele: Concomitant Clinical and Subclinical Varicocele

The reported prevalence of bilateral varicocele with clinical or subclinical right varicocele was variable and believed to be underestimated. A

series of studies discussed in this section illustrates the potential under-diagnosis of bilateral varicocele.

McClure et al. reported in 1986 that using ultrasound increased the diagnosis of bilateral varicocele in infertile men from 6% (clinical) to 70% (clinical and ultrasonic) [6]. In a study on 4075 Turkish boys aged 2–19 years, 0.92% of children (ages 2–10) and 11.0% of adolescents (ages 11–19) had clinical varicocele (grades 1–3). In the adolescent group (n = 2531), only 30 out of 2531(1.2%) had bilateral varicocele detected on physical examination alone, and all right-sided varicoceles were grade 1 [11]. In a German study involving 2756 boys (ages 8–11) and 2008 adolescents (ages 12–18) with clinical examination and Doppler (non-colour) ultrasound, 18.2% boys and 42.8% adolescents were diagnosed to have varicoceles, in which 1.2% boys and 7.2% adolescents had bilateral disease. Among adolescents with clinical varicoceles, there were 16.1% grade 1, 14.0% grade 2, and 8.9% grade 3 left varicoceles; and there were 5.9% grade 1, 0.7% grade 2, and 0% grade 3 right varicoceles. Subclinical varicoceles were reported in only 0.7% boys and 4.5% adolescents. The seemingly low prevalence of subclinical varicocele may be explained by the use of non-colour Doppler ultrasound [12]. In a study of 506 adolescents with clinical left varicocele, 40.3% also had right varicocele (18% subclinical, 10% grade 1, 12% grade 2, and 0.2% grade 3). Therefore, concomitant clinical right varicocele is present in 22.2% of adolescents with left varicocele and up to 40% if subclinical right varicocele is included [13]. Gat et al. reported, in 255 infertile men, left- and right-sided varicoceles were diagnosed on clinical examination in 89.4% and 8.2%, respectively. By using contact scrotal thermography, Doppler ultrasound scrotum, and venography, the diagnoses of left varicocele increased to 97.3%, 98.0%, and 98.4%, respectively; the diagnoses of right varicocele increased to 84.3%, 74.1%, and 82.4%, respectively. Bilateral varicoceles (clinical and subclinical) were diagnosed in up to 80.7% of the patients in this study [5].

A high incidence of bilateral varicoceles reported from the above studies might imply that the majority of right varicoceles were underdiagnosed. In fact, varicocele may occur more commonly on both sides as opposed to a unilateral occurrence.

The Role of Subclinical Varicocele in Fertility

The clinical significance of subclinical varicoceles is uncertain and traditionally not considered an entity that requires attention. In the American Urological Association (AUA) best practice policy and the American Society for Reproductive Medicine (ASRM) practice committee report on varicocele and infertility published in 2001, it is recommended that the optimal method to detect varicoceles is physical examination, and ultrasound should only be used for inconclusive physical examination [3].

There are two randomized controlled trials on treatment effect of subclinical varicocelectomy. Yamamoto et al. randomized 85 men with subclinical varicocele to either surgery (high ligation of internal spermatic vein) or no treatment. The surgery group resulted in significantly better semen parameters but not pregnancy rate [14]. Unal et al. randomized 42 men with subclinical varicocele to medical therapy (clomiphene citrate) or varicocelectomy. Both semen parameters and pregnancy rates were similar in both groups [15].

In a recent meta-analysis on subclinical varicocelectomy by Kohn et al., two randomized controlled trials and 11 retrospective cohorts were analysed [16]. The meta-analysis showed that in men with subclinical varicoceles, treatment (surgery or embolization) resulted in a significant improvement in sperm density (+14 million/mL) versus control (+0.2 million/mL, p = 0.01). However, there was no significant benefit in terms of improvement in sperm motility (treatment group +4.8% vs control group +1.1%, p = 0.43) and annual pregnancy rate (treatment group 15% vs control group 11%, p = 0.49). Therefore, the marked improvement in sperm count did not lead to increase in pregnancy rate in subclinical unilateral varicocelectomy despite improvement in semen parameters [16]. In the part of analysis comparing clinical and subclinical varicocelectomy, better sperm density improvement was observed in clinical varicocelectomy (+17.9 mil-

lion/mL vs 7.8 million/mL, $p = 0.08$) but did not reach statistical significance. Annual pregnancy rates were 12% for subclinical and 18% for clinical varicocelectomy ($p = 0.18$) [16].

The above studies illustrated that treatment of subclinical varicocele alone may improve semen parameters but did not improve pregnancy rates. Although the treatment efficacy on subclinical varicocele alone is doubtful, data demonstrated the potential role of subclinical varicocele repair in augmenting the surgical outcome of repair of clinical varicocele over the contralateral side. The combined effect of bilateral varicocele repair probably surpasses the treatment effect of unilateral repair on either side and may lead to a clinically significant fertility outcome.

Is Varicocele a Progressive Disease?

Delayed family planning is commonly observed worldwide particularly in developed countries. Advanced paternal and maternal age is of concern. While varicocele is common and not all men with varicocele are subfertile, it is uncertain whether untreated varicocele would lead to progressive damage on testicular function and fertility potential over time.

In a British school screening study on 2107 boys between ages 10 and 16, subclinical varicocele was detected in 16.8%. Among a randomly selected group ($n = 36$) in this cohort with subclinical varicocele followed up for 4 years, 5% ($n = 2$) resolved on ultrasound, 67% ($n = 24$) persisted, while 28% ($n = 10$) of them progressed to clinical varicoceles. One out of the ten with varicocele progression had left hypotrophic testis at 4 years. Therefore, a considerable proportion of subclinical varicocele would actually progress, and a small proportion may even lead to testicular hypotrophy [17]. There is also evidence showing higher risk of progression of subclinical varicocele to clinical varicocele in males ages 14–16 years with regular sports activities (36% vs 5%, $p < 0.05$) than in healthy boys without subclinical varicocele [18].

The above studies mainly illustrated the progressive nature of predominant left clinical varicocele. On one hand, the progression of left varicocele may lead to more severe testicular dysfunction bilaterally. On the other hand, it is possible that right varicocele may occur and progress over time and further jeopardize testicular function in the long run.

Is Treatment of Concomitant Right Varicocele Necessary?

The majority of varicocele treatments focus on the left side as it is usually more clinically evident and of higher grade. However, there is growing evidence to support concomitant treatment of right varicocele if detected.

The adoption of bilateral varicocelectomy is highly variable currently. In a review by Woldu et al. (2013) on 15 contemporary series on adult varicocelectomies, a median incidence of 39% bilateral varicocelectomy was observed. The incidence was nevertheless highly variable, ranging from 3.5% to 73.3% for palpable right varicoceles and from 9.2% to 84.6% if subclinical varicoceles were included. In adolescents, the median incidence of bilateral varicocelectomy was only 5% [13].

A study by Amelar and Dubin in 1987 showed that in 41 couples who failed to achieve pregnancy after left varicocelectomy, a subsequently diagnosed right varicocele followed by a sequential right varicocelectomy has resulted in semen improvements in 56% of men and pregnancy in 44% of couples. The authors suggested a previously overlooked right varicocele might explain the initial failure of left varicocelectomy. This illustrated the possible detrimental effect of small or subclinical right varicocele and the benefit of repairing right varicocele as well [19]. Lemack et al., in 1998, showed that in adolescents with large left varicocele and testicular atrophy with concomitant smaller right varicocele, unilateral left varicocelectomy resulted in testicular growth of 50% in the treated side and 23% in the untreated side, while bilateral varicocelectomy resulted in growth rates of 45% over the left side and 39% over the right side [20]. Scherr and Goldstein reported in 1999 that in adult men with grade 2–3 left varicocele and small grade 1 right varicocele, bilateral varicocelectomy resulted in significantly better improvement in semen parameters compared with left varicocelectomy. Motile sperm concentration improved by 95.8%

in the bilateral group versus 42.6% in the unilateral group [21]. This provided evidence for bilateral varicocelectomy in adult men. Gat et al. reported 255 infertile men with varicoceles (left 17.6%, right 1.5%, bilateral 80.8%) diagnosed on Doppler ultrasound and venography. All patients had embolization performed [5]. Ninety percent of right-sided varicocele diagnosed in bilateral cases was subclinical. After treatment (bilateral embolization in 80%), the mean sperm concentration improved drastically from 6 to 21 million/mL, motility from 17% to 36%, and normal morphology from 10% to 17%. The follow-up time ranged from 12 to 42 months, and pregnancy was achieved in 43.5%, and 76% of pregnancies were unassisted. Although there is no control group in this study, the improvements in semen parameters and pregnancy rates were profound. The authors suggested that varicocele may well be a bilateral vascular disease with bypasses or collaterals communicating both sides, and the lack of recognition of bilateral disease and bilateral treatment may explain the persistent abnormal semen parameters and infertility in men after treatment over one side only [5]. Libman et al. compared the effect of bilateral versus unilateral microsurgical varicocelectomy in 369 infertile men. A higher proportion of grade 3 varicoceles was observed in the bilateral group (21% vs 9.6%, $p = 0.16$), and no statistically significant difference in semen parameter improvement between grade 1 and grade 3 varicoceles was observed. The authors reported significantly better improvement in percent motility (8% vs 4.4%) and pregnancy rate (49% vs 36%) in bilateral varicocelectomy group [22]. Bazeem et al. compared unilateral varicocelectomy and bilateral varicocelectomy, and the latter resulted in significantly better sperm concentration improvement, better natural pregnancy rates (48% vs 31%), and less use of artificial reproductive techniques [23].

The majority of the studies on bilateral varicocelectomy were retrospective or non-randomized. Recently, a few randomized studies were published on bilateral treatment for clinical left varicoceles and subclinical right varicoceles. Elbendary and Elbadry included 145 men with left palpable varicocele and right subclinical varicocele in their randomized study. A significantly higher sperm concentration, progressive motility, and natural pregnancy rate (61.6% vs 31.9%, $p = 0.04$) were observed in the bilateral varicocelectomy group compared to the unilateral left varicocelectomy group. The result of the study supported concomitant repair of subclinical right varicocele in addition to correction of left clinical varicocele [24]. However, another study by Zheng et al. did not demonstrate the benefit of bilateral varicocelectomy. In the study, 104 infertile men with left clinical and right subclinical varicocele were randomized to unilateral or bilateral varicocelectomy. Baseline characteristics were similar between the 2 groups, and mean left testis volume was significantly less than right side (12 ml vs 18 ml) in the whole cohort. The improvements in semen parameters and spontaneous pregnancy rate (38% vs 39%) after varicocelectomy were similar in both groups. However, the significant asymmetry in testicular size and left testicular hypotrophy in the study population of a mean age of 32 represented a potential bias. Preoperative testicular hypotrophy in adult men may signify the presence of severe and irreversible testicular damage in this cohort [25]. As a result, the findings of the study may not be generalized to all patients with bilateral varicoceles. It also highlights the importance of patient selection for good surgical outcomes.

While the previous studies yielded conflicting results, a more recently published randomized trial in 2018 represented the largest study to date and may provide us with more insights. Sun et al. randomized 358 men with clinical left and subclinical right varicocele to bilateral versus unilateral microsurgical subinguinal varicocelectomy. Baseline male and female partner ages were around 32 years old. Left testicular volume was slightly lower at 11–12 ml, compared with 13–14 ml in the right side (no *p*-value given). There was 25% grade 1, 50% grade 2, and 25% grade 3 left varicoceles. Significantly higher improvement in sperm concentration (31 vs 25 million/mL), morphology (8% vs 6%), progressive motility (40% vs 34%), and natural pregnancy rate (43% vs 26%, $p = 0.002$) were observed in bilateral varicocelectomy group [7]. Table 30.1 summarizes studies comparing bilateral and unilateral varicocelectomy for palpable left varicocele and low-grade or subclinical right varicocele.

Table 30.1 Studies comparing bilateral and unilateral varicocelectomy for palpable left varicocele and low-grade or subclinical right varicocele

Studies	Design	Varicocele grading	Surgery	Sample size	Semen parameters	Pregnancy rates (natural and Assisted Reproductive Technology (ART))
Scherr and Goldstein (1999) [21]	Non-randomized comparative study	Left: grades 2–3 Right: grade 1	Microsurgical varicocelectomy	Bil 65 Uni 26	Sperm concentration Bil, 24 - > 49 M/mL (+158%) Uni, 41 - > 60 M/mL (+45%), $p < 0.05$ Motile sperm concentration (%) Bil, 12 - > 24 M/mL (+96%) Uni, 20 - > 28 M/mL (+43%), $p < 0.05$	NR
Libman et al. (2006) [22]	Non-randomized comparative study	Bil palpable varicoceles	Microsurgical subinguinal varicocelectomy	Bil 157 Uni 212	Sperm concentration Bil +6.6 M/m Uni +5.8 M/mL, p = NS Motile sperm concentration (%) Bil +8% Uni +4.4%, $p < 0.05$	Natural Bil 49% Uni 36%, $p = 0.049$ ART Bil 13% Uni 14%, $p = 0.96$
Elbendary and Elbadry (2009) [24]	Randomized controlled trial	Left palpable and right subclinical varicoceles	Inguinal varicocelectomy with optical loupes (2.5×)	Bil 73 Uni 72	Sperm concentration Bil 15- > 23 M/mL Uni 15- > 21 M/mL, $p = 0.008$ Motile sperm concentration (%) Bil +14% Uni +3%, $p < 0.001$ Morphology: p = NS	Natural Bil 62% Uni 32%, $p = 0.04$
Zheng et al. (2009) [25]	Randomized controlled trial	Left palpable and right subclinical varicoceles. Significant testicular asymmetry (left 12 ml vs right 18 ml)	Open retroperitoneal approach	Bil 51 Uni 53	Sperm concentration Bil 7- > 24 M/mL Uni 8- > 24 M/mL, p = NS Motile sperm concentration (%): p = NS Morphology: p = NS Recurrence: None at 24 months	Natural Bil 39% Uni 38%, p = NS
Sun et al. (2018) [7]	Randomized controlled trial	Left palpable and right subclinical varicoceles. No testicular asymmetry (left 12 ml, right 13 ml)	Microsurgical subinguinal approach	Bil 179 Uni 179	Sperm concentration Bil 12- > 31 M/mL (+19%) Uni 14- > 25 M/mL (+11%), $p = 0.041$ Progressive motility (%) Bil +17% Uni +12%, $p = 0.041$ Morphology (%) Bil +4% Uni +2%, $p = 0.035$ Recurrence: None at 24 months	Natural: Bil 43% Uni 26%, $p = 0.002$

Bil bilateral; *Uni* unilateral; *NR* not reported; *NS* not significant

Conclusion

The traditional concept of varicocele being a unilateral predominately left-sided disease has its pitfall and is unable to fully explain the overall decline in fertility. The current practice in management of varicocele leads to a lack of awareness of a low-grade or subclinical right varicocele, which may be detected in up to 80% of patients by using Doppler ultrasound. Although treatment for unilateral subclinical varicocele alone is not supported by the current guidelines, the additional detrimental effect of a concurrent right subclinical varicocele in the presence of a clinical left varicocele may be substantial. The fact that varicocele grade may not correlate with the degree of testicular dysfunction and possible progressive nature of varicocele provides the basis to support the potential role of bilateral varicocelectomy. Recent clinical studies demonstrated superior outcomes after bilateral varicocelectomy compared to unilateral repair in patients with clinical left and subclinical right varicoceles. The detrimental effect of unilateral varicocele on bilateral testicular function via increase in intratesticular temperature was also well documented.

In summary, varicocele should be managed as a bilateral disease as supported by the detrimental effect of a unilateral varicocele on both testes and the high prevalence of bilateral varicoceles. Preliminary evidence supports the adoption of bilateral repair in patients with bilateral varicoceles irrespective of grading. Some unanswered questions remain in selecting the best candidates who will benefit from bilateral varicocele repair and the approach of sequential or simultaneous repair.

Review Criteria

An extensive search investigating whether varicocele has bilateral involvement was performed using search engines including ScienceDirect, Ovid, PubMed, and MEDLINE. The study identification was based on the following keywords: "varicocele", "varicocelectomy", and "bilateral". Only articles published in English were considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included.

Multiple Choice Questions and Answers

1. Unilateral clinical left varicocele:
   (a) Is associated with abnormal histology in right testicular biopsy
   (b) Can lead to raised intratesticular temperature on both sides
   (c) May be associated with a right varicocele on imaging
   (d) **All of the above**
2. Which of the following is true?
   (a) **Bilateral varicoceles could be diagnosed in up to 80% infertile men when colour Doppler ultrasound and venogram are used for diagnosis.**
   (b) Up to 40% concomitant clinical right varicocele could be diagnosed in adolescents with clinical left varicocele.
   (c) Grade 2–3 concomitant right varicoceles are the usual findings in the presence of a clinical left varicocele.
   (d) Testicular asymmetry in adolescent is usually defined as more than 50% difference is testicular volume on ultrasound.
3. Concerning subclinical varicocele, which of the following is false?
   (a) The American Society for Reproductive Medicine does not recommend repair of subclinical varicocele.
   (b) Treatment of subclinical varicocele could improve semen parameters according to meta-analyses.
   (c) Treatment of subclinical varicocele could not improve pregnancy rate according to meta-analyses.
   (d) **Varicocelectomy is better than clomiphene in improving semen parameters in subclinical varicoceles.**

4. Subclinical varicoceles in adolescents:
   (a) Never resolve with time
   (b) **Remain as subclinical varicocele in majority of cases**
   (c) Never progress to clinical varicoceles in the future
   (d) Have a lower chance to progress to clinical varicocele with regular sports activities
5. Bilateral varicocele treatment:
   (a) Resulted in better sperm concentration improvement than unilateral repair
   (b) Resulted in better pregnancy rates than unilateral varicocele treatment in most randomized controlled trials
   (c) Was associated with reduced use of assisted reproductive techniques
   (d) **All of the above**

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Why Is Subclinical Varicocele Considered a Different Entity?

31

Mohannad Alharbi and Armand Zini

Key Points

- Left subclinical varicocele in children is a risk factor for the progression to a clinically detectable varicocele and should be viewed as a pathological condition.
- Scrotal ultrasound with color Doppler imaging (CDUS) has become the most widely used method for detecting subclinical varicocele because of its high sensitivity and specificity.
- The most widely accepted CDUS criteria for subclinical varicocele diagnosis is when one or more veins have a diameter >3 mm, at rest or during Valsalva maneuver.
- There is very little evidence to support medical treatment of subclinical varicocele.
- Additional prospective and randomized controlled trials are needed to determine the value of repairing subclinical varicocele in infertile couples.

Introduction

Subclinical varicocele is considered by some authors to be a pathological condition that may progress to a clinical varicocele, and it has a detrimental effect on spermatogenesis, which justifies the surgical repair. On the other hand, other studies did not show a beneficial effect of repairing subclinical varicocele. ASRM and AUA made clear statement to resolve this controversy. Details about the studies that addressed this controversial topic are demonstrated in the following sections.

Definition

In 1960, Ivanissevich wrote that reflux of blood into the internal spermatic vein is an important feature of varicocele, with physical exam being the only method to diagnose varicocele [1, 2]. In 1966, Ahlberg et al. [3] introduced spermatic venography as a method that allowed diagnosis of varicocele based on documentation of distinct retrograde flow in the internal spermatic vein. This retrograde flow can also occur in the absence of clinical or physically palpable varicocele and has been labelled subclinical varicocele [2].

Most would agree that a subclinical varicocele is an abnormality of testicular blood flow characterized by instrumentally measurable venous

M. Alharbi (✉)
Division of Urology, Department of Surgery, McGill University, Montreal, QC, Canada

Department of Urology, College of Medicine, Qassim University, Qassim, Saudi Arabia

A. Zini
Division of Urology, Department of Surgery, McGill University, Montreal, QC, Canada

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reflux detected by a variety of diagnostic tools (e.g., color Doppler ultrasound) in the absence of clinically detectable varicocele [4].

Prevalence

Few studies have reported on the prevalence of subclinical varicocele. One of the earliest reports was published in 1987 by Kursh et al. [5]. These authors examined 100 fertile men presenting for vasectomy and found that 44% of the men had subclinical varicocele by Doppler stethoscope. The possible explanation for this high prevalence was likely related to the detection method. Two subsequent smaller series reported a prevalence ranging from 17% to 24% [6, 7]. In 1999, Zini et al. performed a retrospective study of 404 patients presenting for infertility evaluation and found that 10% had subclinical left varicocele and 10% had bilateral subclinical varicocele diagnosed by scrotal ultrasonography [8].

In children and adolescents, one of the areas of particular interest is the natural history of subclinical varicocele. Cervellione et al. performed a school screening of 2107 boys aged 10–16 years in Italy [9]. They reported that 16.8% had subclinical varicocele. They selected 36 children of the 16.8% with subclinical varicocele and followed them annually for 4 years. They observed that 28% of the boys' subclinical varicocele progressed to a clinically detectable varicocele [9]. In a similar study, Yu et al. found that up to 10% of adolescents developed a clinically evident varicocele after an earlier presentation with subclinical varicocele [10]. Interestingly, only patients with a left subclinical varicocele were found to progress and later developed a clinically detectable left varicocele (none of the patients with a right subclinical varicocele progressed to later develop a clinically evident right varicocele) [10].

The abovementioned studies suggest that left subclinical varicocele in children is a risk factor for the progression to a clinically detectable varicocele and should be viewed as a pathological condition [9]. Altogether, the data indicate a low likelihood of resolution of left subclinical varicocele, a high rate of its progression to a clinically evident one, and a potentially negative impact of subclinical varicocele in adults (based on an associated decrease in ipsilateral testicular volume) [8, 9]. For these reasons, some have proposed to follow children with subclinical varicoceles (particularly, if left sided) with annual physical exam and ultrasound to detect a clinical varicocele [8–10]. Studies evaluating the natural history and progression of subclinical varicocele in adults are lacking [9].

Diagnosis

Physical examination is critical in the diagnosis of clinical varicocele. However, its specificity is only 70% [11]. The introduction of imaging studies has led to improved detection and characterization of varicocele, especially subclinical ones.

Venography is the accepted gold standard for diagnosis of subclinical varicocele [12]. Venography has a very high (nearly 100%) sensitivity, but its invasive nature, cost, and morbidity limit its use [13]. Scrotal thermography and radionuclide scanning have emerged as non-invasive alternatives to venography, and these techniques are based on detecting hyperthermia, blood pooling, or reflux of tracer but with some limitations including lack of specificity, wide variability, and absence of agreement on standard diagnostic criteria [14].

Scrotal ultrasound with color Doppler imaging (CDUS) has become the most widely used method for detecting subclinical varicocele because of its reported high sensitivity and specificity (97% and 94%, respectively), non-invasiveness, availability, and low cost [12, 14]. Diagnosis of varicocele by CDUS is based on demonstration of blood reflux in the internal spermatic veins and venous distension [12]. Although there are no standardized criteria for diagnosis of subclinical varicocele by CDUS, investigators have generally suggested the following two criteria: (1) a 2–3 mm cut-point for maximum venous diameter and/or (2) presence of retrograde flow [14]. Additional refinements of these criteria have been proposed by some to diagnose subclinical varicocele by CDUS: mea-

surement of scrotal vein diameter in upright and supine position, measurement of change in venous diameter during Valsalva maneuver, sum of venous diameter, flow volume, retrograde flow duration, and change of flow during inspiration [11, 15]. Studies suggest that venous diameter is the most accurate value for subclinical varicocele diagnosis when compared to other criteria. Interestingly, Hoekstra et al. showed that scrotal veins <2.5 mm are never palpable, whereas veins >3.5 mm are palpable and indicate clinical varicocele [16]. Another study by Eskew et al. observed that the maximum vein diameter for subclinical varicocele was 3.6 mm [15, 17]. Multiple scoring systems have been used for varicocele diagnosis and grading: Sarteschi [18] and Dubin [19] classifications and Chiou et al. scoring [20].

The most widely accepted CDUS criteria for subclinical varicocele diagnosis is that proposed by McClure and Hricak. McClure and Hricak proposed that the diagnosis of subclinical varicocele can be made when one or more veins have a diameter >3 mm, at rest or during Valsalva maneuver [6, 8]. Others have proposed that the diagnosis of subclinical varicocele is established when (1) at least 2 veins are at least 2 mm in diameter and (2) retrograde flow or a change in vein diameter is observed when comparing at rest and Valsalva maneuver [15]. Furthermore, Pilatz et al. suggested that clinical varicocele can be predicted using venous diameter of >2.45 mm at rest or >2.95 with Valsalva maneuver in supine position [11].

Effect on Fertility

Mechanism

Various theories have explained the association between subclinical varicocele and impaired spermatogenesis. The most widely known one is elevated scrotal temperature, in which there is a disruption in the countercurrent heat exchange system [21]. The reflux of renal and adrenal metabolites can disturb spermatogenesis [22]. The reflux theory has been proposed to be the likely reason for impaired spermatogenesis in subclinical varicocele [23]. Another mechanism is venous stasis which results in hypoxia and toxic metabolite accumulation. Moreover, subclinical varicocele can negatively impact testicular volume with resultant Sertoli and Leydig cell dysfunction [8].

More recently, two studies have identified pathophysiological changes in men with subclinical varicocele. García-Peiró et al. found that infertile patients with subclinical varicocele have substantial sperm DNA fragmentation, with levels that were similar to infertile men with clinical varicocele [24, 25]. Abo El-khair et al. observed decreased semen parameters and increased semen leukocytes in infertile men with subclinical varicocele [26]. Furthermore, increased expression of fractalkine (which positively correlates with DNA fragmentation and in turn induces reactive oxygen species (ROS)) and semen oxidative stress has been found in infertile men with subclinical varicocele [26]. Collectively, these observations suggest that infertile men with subclinical varicocele have impaired spermatogenesis and semen quality as a result of testicular and/or semen oxidative stress [26].

Predictors

There is no consensus regarding the effect of subclinical varicocele on testicular volume. Some studies reported no significant effect on testicular volume by subclinical varicocele [15, 27]. On the other hand, Zini et al. have shown that left subclinical varicocele is associated with decreased left testicular volume [8].

It has been reported that high sperm DNA fragmentation and advanced paternal age were predictors for subfertility in patients with subclinical varicocele [26]. Furthermore, Chen conducted a study on 150 patients with subclinical varicocele to determine the predictive factors for subfertility [28]. He found that peak retrograde flow (PRF) >29 cm/sec, resistive index (RI) >0.55 ml/s, pulsatility index (PI) >0.99 ml/s, total testicular volume <27 cc, and scrotal temperature >34.9 °C were predictive factors for subfertility in subclinical varicocele patients [28].

On the contrary, Akcar et al. demonstrated that subclinical varicocele was not associated with a decrease in testicular volume and there was no effect on intra-testicular arterial RI [15].

Treatment

Indications for Repair

Subclinical varicocele has been viewed as a condition distinct from clinical varicocele [4]. To date, the role of subclinical varicocele repair in infertile patients is controversial. The Practice Committee of the American Society for Reproductive Medicine (ASRM) and the American Urology Association (AUA) recommend against repairing subclinical varicocele [29, 30]. However, there is some evidence in support of subclinical varicocele repair in pediatric patients with testicular asymmetry and in men with left clinical and right subclinical varicocele [31]. In the following sections, we will discuss the available evidence for and against subclinical varicocele repair.

The Role of Medical Therapy

There is very little evidence to support medical treatment of subclinical varicocele, although two medications have been tested in this context: clomiphene citrate and bioflavonoids [32].

Unal et al. conducted a prospective randomized study comparing varicocelectomy to a control arm receiving clomiphene in patients with subclinical varicocele [32]. Their aim was to investigate the effect of these interventions on sperm parameters and pregnancy rates. They found that varicocelectomy improved sperm concentration and motility significantly compared to clomiphene citrate. On the other hand, there was no statistical significant difference between varicocelectomy and clomiphene citrate in regard to pregnancy outcomes [32]. Nonetheless, they concluded that their results did not resolve the dilemma of subclinical varicocele treatment.

The other treatment that has been examined in men with subclinical varicocele is the use of bioflavonoids [4]. It has been proposed that during stasis and hypoxic states, bioflavonoids protect the venous endothelial cells by diminishing the drop in adenosine triphosphate, and this results in reducing the inflammatory response, attraction of neutrophils, venous damage, and growth factor release [4]. These molecular changes would otherwise exacerbate venous insufficiency [4]. Zampieri et al. evaluated 168 adolescents aged 10–14 years with left subclinical varicocele [4]. The treatment group received bioflavonoids for 3 months (n = 73) and was compared to an untreated cohort (n = 95). They evaluated changes in testicular volume, evolution of vein reflux on Doppler, and progression to clinical varicocele over the short- (3–6 months) and long-term (1–4 years) period [4]. Their long-term findings showed stabilization of vein reflux (47% vs. 38%, $P < 0.05$), lower rate of progression to clinical varicocele (11% vs. 31%, $P < 0.05$), and higher rate of spontaneous resolution (41% vs. 31%, $P > 0.05$) in the treatment group compared to the control group. However, bioflavonoids did not prevent the need for surgery that is related to testicular growth arrest [4].

Effect of Subclinical Varicocele Repair on Semen Parameters and Pregnancy Outcomes

One of the first studies on subclinical varicocele repair was reported by Greenberg et al. in 1979. These investigators published their preliminary results on 19 patients with subclinical varicocele, of which 5 had oligoasthenozoospermia. These men underwent internal spermatic vein ligation, resulting in improvement in semen parameters in two patients who later fathered children [33]. In 1985, Marsman studied 58 patients with clinical and subclinical varicocele [34]. He observed an improvement in semen parameters after embolization of internal spermatic veins and reported that some of the couples that underwent subclinical varicocele embolization achieved a pregnancy [34].

A number of studies have reported on the outcomes of subclinical varicocele repair by percutaneous embolization. Yarborough et al. followed 13 patients post-embolization of subclinical varicocele and found an improvement in sperm concentration but no change in sperm morphology or motility [35]. Of note, they reported one pregnancy with subsequent miscarriage in their series [35]. In 1995, Marsman compared the results of 46 patients with subclinical varicocele to 40 patients with clinical varicocele post-embolization and observed an improvement in all semen parameters in both groups with a pregnancy rate of 39% in the subclinical varicocele group and 42.5% in the clinical varicocele group [2]. They suggested that future studies should be designed as controlled trials, specifically comparing outcomes of subclinical varicocele patients after treatment vs. no treatment [2]. More recently, Cantoro et al. applied the design suggested by Marsman [2] in their study [36]. They performed a prospective study of subclinical varicocele patients: 218 patients underwent embolization (treatment group), and 119 underwent no treatment (observation group) [36]. The improvement in sperm concentration and motility was significantly greater in the treatment group. Moreover, the pregnancy rate was significantly higher in the treatment group compared to the no treatment group (46.3% vs. 11.8%, respectively, $P < 0.05$) [36].

Several surgical series have demonstrated variable outcomes following repair of subclinical varicocele. McClure et al. evaluated 56 infertile patients, 38 with clinical varicocele and 18 with subclinical varicocele [37]. Surgical varicocelectomy was associated with improvement in motility (but not in concentration) in both the clinical and subclinical varicocele groups [37]. Dhabuwala et al. operated on 38 patients with clinical varicocele and 16 patients with subclinical varicocele and reported an improvement in sperm parameters in both groups after varicocelectomy with pregnancy rates of 47% and 50%, respectively [23]. Jarow et al. observed a suboptimal response to varicocelectomy in subclinical varicocele patients compared to patients with clinical varicocele in terms of semen parameters [12]. Interestingly, they found that varicocele grade was associated with sperm parameter improvement after varicocelectomy, with larger varicocele having a better semen improvement after surgery compared to the small grades [12]. The main weaknesses of the previously mentioned studies were lack of a proper untreated control group and absence of pregnancy data in some series [36, 38].

Yamamoto et al. performed a prospective randomized controlled study of 85 patients diagnosed with subclinical varicocele by scrotal thermography [39]. They randomly assigned 45 patients to undergo high ligation of internal spermatic veins (group 1) and 40 patients to no treatment (group 2) and reported some effect on sperm parameters in favor of group 1. However, there was no statistical difference in pregnancy rates between the two groups [39]. Seo et al. assessed 143 patients with left subclinical varicocele and offered them surgery or medical treatment, and patients who refused any treatment were included as a control group [40]. The three groups were treated as follows: 25 patients underwent microsurgical varicocelctomy (group 1), 93 patients agreed to medical treatment with L-carnitine (group 2), and 25 patients undergoing no treatment served as a control group (group 3) [40]. They noted a significant improvement in sperm concentration in the surgery group with no improvement in the other sperm parameters. On the other hand, no improvement in semen parameters was seen in the L-carnitine group [40]. Pregnancy rate was significantly higher in the surgery group compared to the L-carnitine and control groups (60%, 34.5%, 18.7%, respectively, $P < 0.05$) [40]. They concluded that microsurgical varicocelectomy is an ideal option for patients with subclinical varicocele [40].

A recent systematic review and meta-analysis on the impact of subclinical varicocele repair on fertility was reported by Kim et al. [41] These investigators included seven trials with a total of 548 patients, and they observed an improvement in forward progressive motility after subclinical varicocelectomy. However, no statistically significant difference in other sperm parameters was observed [41]. Moreover, there was no significant

benefit of varicocele repair on pregnancy rates (odds ratio = 1.29, 95% CI 0.99–1.67).

Left Clinical and Right Subclinical Varicocele: Unilateral vs. Bilateral Repair

The outcomes of bilateral versus unilateral varicocele repair in men with left clinical and right subclinical varicocele are inconsistent. Some studies have shown a beneficial effect of bilateral compared to unilateral repair. However, other reports did not find any difference between the two approaches in terms of sperm parameters and pregnancy outcome.

One of the earliest reports on the repair of right subclinical varicocele was published by Kondoh et al. in 1993. These investigators evaluated the outcomes of 56 infertile men aged 26–41 years and divided them into two groups: group 1 having 30 patients with left clinical and right subclinical varicocele and group 2 having 26 patients with left clinical varicocele only [42]. After performing left varicocelectomy in all patients, they observed a significantly greater improvement in sperm count and motility in group 1 compared to group 2. They suggested that repair of right subclinical varicocele may be beneficial [42].

Pasqualotto et al. evaluated 50 patients: 30 men with left clinical varicocele (group 1) and 20 with left clinical and right subclinical varicocele (group 2) [43]. Group 1 underwent unilateral varicoclectomy, whereas group 2 underwent bilateral varicocelectomy (microsurgical subinguinal or inguinal approach). Postoperatively, they noted a significantly greater improvement in sperm concentration in group 2 compared to group 1. However, sperm motility did not change significantly in the two groups. They also reported a higher pregnancy rate in group 2 compared to group 1 (66.7% vs. 33.3%, respectively, $P < 0.05$) [43].

Elbendary et al. performed a prospective randomized study in oligoasthenozoospermic men with left clinical and right subclinical varicocele [44]. They included 73 patients in the first group who underwent bilateral inguinal varicocelectomy (group 1) and 72 patients in the second group who underwent left inguinal varicocelectomy using optical loupes magnification in both groups. They observed a significantly greater improvement in sperm concentration and motility in patients who underwent bilateral varicocelectomy compared to patients in the second group with a significantly higher pregnancy rate in the former group (62% vs. 32%, respectively, $P < 0.05$) [44]. More recently, similar results have been reported by Sun et al. [45] These authors conducted a prospective randomized controlled study of 358 patients with left clinical and right subclinical varicocele treated with either bilateral ($n = 179$) or unilateral ($n = 179$) microsurgical subinguinal varicocelectomy [45]. These investigators observed that both bilateral and unilateral microsurgical subinguinal varicocelectomies were associated with improved semen parameters. However, the group treated by bilateral varicocelectomy experienced a significantly greater improvement in semen parameters than the unilateral varicocelectomy group. Moreover, the clinical pregnancy rate was higher in the bilateral varicocelectomy group than the unilateral varicocelectomy group (42.5 vs. 26.0%, respectively) [45].

In contrast, other studies did not show an advantage of bilateral over unilateral repair in sperm parameters and pregnancy rates. Zheng et al. randomly assigned men with left clinical and right subclinical varicocele to bilateral ($n = 51$) or unilateral ($n = 53$) varicocele repair. They found that both bilateral and unilateral varicocelectomies were associated with improved semen parameters. However, there was no difference in postoperative sperm values and pregnancy rate in the bilateral and unilateral repair groups [46].

The observed differences in the previously mentioned studies could be related to a number of factors. First, variable cut-off definitions have been used to diagnose subclinical varicocele. Second, different surgical approaches were used which can impact the observed results. Finally, the inconsistency in the study designs and the inherent variability in the semen analysis may be

possible explanations [41, 45]. At this time, additional prospective and well-designed studies with larger samples are needed to resolve this debate.

Conclusion

In summary, the data indicate that a subclinical varicocele may be a pathological condition and may predispose to the development of a clinical varicocele in adolescents. To date, the available literature demonstrates that there is insufficient evidence to support varicocele repair for the treatment of infertile couples with a left subclinical varicocele. Moreover, correction of right subclinical varicocele in the context of left clinical varicocele remains controversial [38, 41]. Additional prospective and randomized controlled trials are needed to determine the pathophysiology of subclinical varicocele and the value of repairing this condition in infertile couples.

Review Criteria

An extensive search was performed including articles from 1952 to 2018. The PubMed and MEDLINE search terms included "subclinical varicocele," "varicocelectomy," and "infertility." The main focus was on studies discussing the prevalence of subclinical varicocele, the diagnosis of subclinical varicoceles, and the evidence behind its repair.

Multiple Choice Questions and Answers

1. In a study that examined 100 fertile men presenting for vasectomy, the author found the prevalence of subclinical varicocele to be:
   (a) 20%
   (b) 50%
   (c) 35%
   (d) **44%**
2. The most common criterion for subclinical varicocele diagnosis is:
   (a) **Venous diameter**
   (b) Flow volume
   (c) Retrograde flow duration
   (d) Change of flow during inspiration
3. The most likely mechanism for impaired spermatogenesis in subclinical varicocele is:
   (a) Scrotal hyperthermia
   (b) Venous stasis
   (c) **The reflux theory**
   (d) Heat shock proteins
4. The main weakness of the early studies regarding the effect of subclinical varicocele repair on semen parameters is:
   (a) Small sample size
   (b) **Lack of a proper untreated control group**
   (c) Underpowered
   (d) Presence of confounding variables
5. Which of the following is true regarding the systematic review done by Kim et al. [35] addressing the effect of subclinical varicocelectomy on fertility?
   (a) **They observed an improvement in forward progressive motility.**
   (b) They observed an improvement in sperm concentration.
   (c) They found a benefit of varicocele repair on pregnancy rates.
   (d) No change in sperm parameters was noted.

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Why Is It That Not All Men with Varicocele Are Infertile?

32

Ramy Abou Ghayda and Martin Kathrins

Key Points

- The assumption that varicocele is an essential contributor to the etiology of male factor infertility is still subject to debate in many aspects, and more than half of males with varicocele retain their fertility potential.
- Presence of a varicocele does not automatically equate to impaired fertility or altered semen parameters.
- Post-varicocelectomy outcomes are still subject to discussion especially regarding their weak evidence and questionable clinical significance. Thus, the role of surgical correction of varicocele in fertile patients is still debatable and equivocal. Female factor may play a major role in a couple's fertility regardless of the status of varicocele.
- New markers, such as proteomics and inhibin B, provide an objective and individualized approach to patients with varicocele by ultimately providing specific evidence of varicocele-induced injury.
- Genetic and epigenetic variation among individuals may explain why some men are protected and less susceptible to the negative pathophysiological effects of varicocele.

Introduction

The assertion of varicocele as one of the most common causes of male infertility has repeatedly been quoted in most urologic references. Varicocele is considered the second most common cause of male infertility after idiopathic infertility. It is also considered the most common correctable cause of male infertility. The large number of research studies focusing on this causal effect has not yet solved the confusion, and till this date, the debate has not reached a scientifically definitive conclusion. Moreover, the literature has failed to identify a definitive strong association between fertility potential and the presence of a varicocele. This may be due to the lack of strong correlation between varicocele and male infertility, or between varicocelectomy and improved pregnancy outcomes. On the other hand, many studies have demonstrated that not all men with varicocele

R. Abou Ghayda (✉)
Department of Urology, Brigham and Women's Hospital, Boston, MA, USA
e-mail: raboughayda@hsph.harvard.edu

M. Kathrins
Department of Urology, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, USA

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are infertile. In fact, a majority of them retain their fertility potential despite being diagnosed with a varicocele. This observation has led to the idea that only a specific subgroup of men is negatively affected by this pathology. These men may have been predisposed to reproductive dysfunction or more susceptible than others to the pathophysiologic changes triggered by the presence of varicocele.

Varicocele Is Also Present in Fertile Men

The prevalence of varicocele in the infertile population has been widely studied. According to a World Health Organization (WHO) report, 25.4% of men, with abnormal semen parameters, seeking fertility care were found to have a varicocele [1]. Other references reported a higher prevalence of 35% among this group of men [2].

However, varicocele is also present in the general population and a good percentage of the fertile sample of men. Early studies have reported that it is present in at least 15% in the general postpubertal male population and 11.7% of men with normal semen parameters [1, 3]. It has been reported that 61% of men desiring vasectomy had clinical and subclinical varicocele [4]. In his review, Zini et al. found that 75% of men with varicocele were fertile [5].

Varicocele Does Not Necessarily Lead to Male Infertility

Many references suggest that varicocele is an established etiology for male infertility; however, this relationship remains unclear. Studies have proven that two thirds of the men with varicocele retain their fertility [6, 7]. Additionally, reports on varicocelectomy did not always lead to an improvement in fertility potential [8, 9]. Scientists who challenge the causality of varicocele on male infertility argue that the basis of the initial claim is the high rate of varicocele detection in infertile men compared to the general population, three times more in some studies [9, 10].

Table 32.1 Is varicocele and male infertility truly related?

Study	Year	n/population	Infertility risk
Uehling et al. [16]	1968	776/ military	No significant difference between patients with or without varicocele
Thomason et al. [35]	1979	909/routine well-being clinic	Presence of varicocele was independent from fertility
Pinto et al. [48]	1994	946/routine urologic clinic	Testicular volume was not significantly different between fertile and infertile males with varicocele and testicular atrophy was independent of fertility
Lund et al. [34]	1998	68/military	Paternity and fertility were not statistically different between males with and without varicocele
Safarinejad et al. [49]	2007	11,441/ routine clinic	Higher risk of infertility in patients with varicocele

Based on data from Ref. [5]

Examiners' bias in diagnosing varicocele by physical examination has been studied extensively showing a great variability and heterogeneity in its detection rate [10] (Table 32.1).

Varicocele Is Not Always Linked to Detrimental Semen Analysis

Many studies have failed to prove that the presence of varicocele negatively affects semen parameters. In a group of almost 600 fertile men on their pre-vasectomy visit, De Castro et al. found no difference in semen parameters between men with or without varicocele [11]. In a group of sperm donors, hormonal and seminal parameters were comparable between the two groups [12]. During a routine military physical exam, Zargooshi et al. reported no association between incidental varicocele finding and semen parameters associated with infertility [13].

Further studies have repeatedly shown that many patients with varicocele have normal semen

parameters and that majority of patients with varicocele were able to father children [14–16].

Lipschultz et al. repeatedly compared semen analysis in men with and without varicocele and showed no clinical or statistical difference in the different semen parameters between infertile men with or without varicocele [17]. Other studies have also confirmed these findings, stating that semen parameters are abnormal in infertile men, regardless of the presence or absence of varicocele [18]. Instead, abnormal semen analysis may be related to the presence of an infertility status.

Why a Subgroup of Men Has Preserved Fertility Despite Having a Varicocele: Markers for Varicocele-Induced Injury

These biomarkers will give hope to stratify individuals with varicocele into susceptible and non-susceptible, those whose fertility might be affected and those who were inherently protected. They will provide the physicians with objective tools and measurements to assess the effect that varicocele has on fertility and direct diagnostic and therapeutic interventions accordingly.

Sperm Apoptosis

Although many studies have shown an increase in germ cell apoptosis in testicle with varicocele, others did not. Fujisawa et al. studied 56 testicular biopsies from patients with clinical varicocele and compared them to a healthy control group. The authors demonstrated a decrease apoptotic index in testes of varicocele patients compared to healthy control. They also showed a decrease in apoptotic cells per Sertoli cells compared to normal men. They also did not find a correlation between the apoptotic index and semen parameters or reproductive hormone levels [19].

The pathophysiologic mechanism relating varicocele to ejaculated sperm and testicular germ cell apoptosis is controversial and poorly understood. Chen et al. described a possible compensatory effect between the decreased apoptosis in the testicle compared to its increase in the ejaculated specimen in patients with varicocele. Even though sperm apoptosis might play a role in varicocele-related fertility, studies have repeatedly shown that sperm apoptosis does not correlate with semen parameters or spermatozoa kinematics [20]. The findings of normal semen quality and kinematics despite the increased apoptosis in ejaculated sperm of varicocele patients have contributed to the belief that a subgroup of men have protective and compensatory mechanism for any potential insult caused by the varicocele. The fertility of these men is therefore preserved despite the presence of a clinical varicocele.

Proteomics

To better understand the varicocele-induced infertility, researchers have attempted to study the semen profile of an affected individual at the molecular levels. Proteomics has emerged as a recent assessment tool of proteins that might be the culprit in mechanisms leading or contributing to male infertility.

In their review, Agarwal et al. reviewed studies that used proteomic analysis to compare high oxidative stress state of infertile men, in seminal plasma and spermatozoa, compared to normal fertile subjects. They were able to isolate multiple expressed proteins that might play a role in the response and impediment of oxidative stress. The authors also listed a proposed list of suggestive biomarkers for the inflammatory and oxidative stress state [21].

Agarwal et al., in another paper, studied the proteomics of spermatozoa explicitly in infertile men with varicocele. They aimed to identify specific proteins expressed in varicocele-related infertility irrespective of other clinical variables. The study was able to identify five biomarkers and validate their proteomic expression; they include PKAR1A, AK7, CCT6B, HSPA2, and ODF2. This study contributed further to the growing field of fingerprinting assessment of men with varicocele based on molecular param-

eters and gave a reliable instrument to measure how much varicocele are truly affecting the fertility potential of men with this condition [22].

Oxidative Stress and Antioxidants

Many hypotheses have been proposed for varicocele-impaired fertility. One of these assumptions lay out the etiology of impaired glycolysis leading to intracellular acidification and inefficiency in the protective antioxidant system. The subsequent increase in reactive oxygen species (ROS) will result in distorted membrane architecture, altering sperm function and morphology. In addition, the resulting lowered pH levels further diminish sperm motility [23]. However, the authors clearly state that some men with varicocele are less susceptible to these notorious changes. The inter-individual genetic and epigenetic variability makes the previously proposed mechanism not universally applicable. Some individuals demonstrate a less restricted glucose reserve and more effective accessory glands. Hence, ROS accumulation and lower intracellular pH in these patients are not as notorious to sperm as projected. Fertility potential is therefore preserved despite the presence of varicocele [24].

In their study, Abd-Elmoaty et al. clearly demonstrated that patients with varicocele have higher levels of oxidative stress and diminished levels of protective antioxidants in their semen. According to the authors, this dual imbalance will lead to a detrimental outcome regarding the semen parameters and indirectly affect negatively the fertility potential of patients. Interestingly, this disproportion was also established among fertile men with normal semen parameters [25]. On the other hand, reports, such that of Zargooshi et al., have demonstrated that a majority of patients with high-grade varicocele were found to have normal sperm parameters. They also stated that varicocele grade was not associated with worst semen morphology or function [26]. It can thus be concluded that the insult mechanisms induced by varicocele are incremental and additive in nature. They are not an all-or-none process. Although the presence of varicocele in certain individuals might lead to pathological changes in semen parameters leading to infertility, these changes should reach a certain physiologic threshold to clinically alter the fertility potential.

Inhibin B

One of Sertoli cells' paracrine function might give insight on why not all men with varicocele are infertile. Inhibin B is a dimer produced by the testis, predominantly by Sertoli cells. Inhibin B is responsible for spermatogenesis regulation through the feedback loop on FSH secretion. Many studies have evaluated the level of inhibin B in patients with varicocele. The level of this latter glycoprotein was inversely correlated with spermatogenesis that is negatively affected by the presence of varicocele [27]. Varicocelectomy in patients whose varicocele is notoriously affecting their fertility has proved to be effective in restoring normal levels of inhibin B in the postoperative period. This increase has been proved to positively correlate with testicular function [28]. Therefore, inhibin b has the potential to be used as an effective marker on the effect of varicocele on testicular function, directing the therapeutic decisions [29]. A normal level of inhibin B in patients with varicocele might indeed point towards a normal spermatogenesis and a subsequent intact fertility.

Varicocelectomy Might Not Affect Fertility Outcomes

Theoretically, if varicoceles are at the heart of etiologies of male infertility, their surgical correction should lead unequivocally to improvement in semen parameters and pregnancy rates. First, large prospective or randomized trials to determine the gold standards of varicocele surgical approach are still lacking. Some studies have demonstrated that varicocelectomy may improve semen parameters, but that does not translate into

Table 32.2 Major post-varicocelectomy randomized control trials and their reported effect on pregnancy rate

Study	Type of varicocele	Improvement in seminal parameter	Improvement in pregnancy rate
Madgar et al.	Clinical	Concentration, motility, and morphology	Positive
Krause et al.	Clinical	Negative	Negative
Nilsson et al.	Clinical	Negative	Negative
Breznik et al. [36]	Clinical	Negative	Negative
Nieschlag et al. [30]	Clinical	Concentration	Negative
Yamamoto et al.	Subclinical	Concentration	Negative
Grasso et al.	Subclinical	Concentration	Negative
Unal et al.	Subclinical	Concentration and motility	Negative

Based on data from Ref. [14]

higher pregnancy rates [30, 31]. Studies reporting a favorable effect on semen parameters following a varicocelectomy are mostly uncontrolled. Additionally, an extensive review of the literature, including but not limited to those performed by Evers et al. and Kamischke et al., concluded that the current data is inconclusive and not supportive of the role of varicocelectomy in improving semen parameters and pregnancy rates in a clinically significant manner [32, 33]. Hence, patients with varicocele may not necessarily benefit from a varicocelectomy, and these patients may retain their fertility potential regardless of any therapeutic intervention.

Varicocelectomy is Not Always Correlated with Positive Pregnancy Outcomes

The most significant primary outcome of any fertility intervention is pregnancy rates. Unfortunately, studies with pregnancy rate outcome are hard to randomize and control as they are affected by many confounding variables. Studies have been conducted showing conflicting findings. Some of the many studies showing no correlation between being diagnosed with a varicocele and fathering a living child include those by Uehling et al. and Thomason et al. Lund et al. even conducted a prospective trial over 8 years but failed to show a difference in paternity rates between control groups and men with varicocele [16, 34, 35]. Nieschlag et al. did one of the first, well-designed, randomized controlled trials on the matter. After 1 year of follow-up post-varicocelectomy, they reported similar pregnancy rates to those subjects with clinical varicocele who elected no intervention [30]. Breznik et al. also confirmed the non-superiority of varicocelectomy over observation in improving pregnancy rates [36] [Table 32.2]. In most of the studies, varicocele was not the culprit of the infertility. Rather, female factors, such as advanced maternal age, were more significant risk factors for the observed couple's infertility.

Varicocelectomy Does Not Always Improve Semen Analysis

Many studies have attempted to examine the effect of a varicocele repair on semen analysis. An early meta-analysis in 2007 by Agarwal et al. evaluated the effect of varicocele on semen parameters. They reported a statistically significant increase in sperm concentration and motility after inguinal microsurgical varicocelectomy [37]. Moreover, 36% of couple with low seminal parameters and who satisfy the definition of infertility are capable of natural conception [38]. This leads us to the conclusion that among patients who underwent varicocelectomy as an infertility intervention, a portion of the positive correlation might be inaccurately credited to the surgical intervention. Overall, the studies on the effect of varicocelectomy on semen parameters are not homogeneous. Furthermore, significant flaws exist in the majority of studies, making them hard to generalize and adapt [Table 32.2].

Varicocele Does Not Always Lead to Oxidative Stress and Altered Sperm DNA Integrity

Oxidative Stress

Superoxide anions, the hydroxyl radical, nitrous oxide, and hydrogen peroxide are examples of reactive oxygen species (ROS). Their presence is essential in cellular signaling, facilitation, and regulation. Usually, the production of these ROS and their neutralization through scavengers and antioxidants are tightly regulated. Their deregulation leads to oxidative stress (Fig. 32.1).

Sakamoto et al. in their retrospective study and Chen et al. in their prospective study measured levels of oxidative stress markers and found a statistical decrease in their levels following varicocelectomy [39, 40]. However, it is interesting to note that the oxidative stress of varicocele was demonstrated in both infertile and fertile subjects [41]. Another study by Cocuzza et al. found no difference in ROS in fertile men with varicocele compared to fertile subjects without varicocele. The detected levels were independent of varicocele grade of testicular volume [42].

Sperm DNA Integrity

In 2013, the American Society for Reproductive Medicine (ASRM) published their guidelines. The committee advised against the assessment of DNA damage, as the current methods for assessing sperm DNA integrity are neither homogeneous nor standardized, and the results they are generating are not correlated to reproductive outcomes [43]. Despite the ASRM recommendation, sperm DNA integrity and its relation to varicocele have gained popularity among clinicians. However, studies relating the effect of varicocele on DNA fragmentation and if some beneficial outcomes do exist after a varicocelectomy are still lacking. Macini et al. showed no change in sperm DNA fragmentation after surgical repair [44]. Another study by Bertolla et al. found more DNA damage in patients with varicocele. However, the semen analysis between the study group and the control showed no difference [45]. Because of the highly variable and non-standardized DNA fragmentation tests, evidence at this point is weak in providing exact measurements. DNA fragmentation might be an important tool in the future in assessing and directing therapies. To be clinically viable and applicable, assays and reporting should head towards standardization and reproducibility [46, 47].

Conclusion

Since the earliest history up until the current times, varicocele has been linked to male infertility. Still, this relationship is subject to an extensive debate that is not yet resolved. We have reviewed in the preceding discussion a significant number of scientific references challenging the causality association between the presence of varicocele and male infertility. Contrary to the overwhelming beliefs, not all men with varicocele are infertile. Available evidence suggests that studies conducted in this regard are flawed,

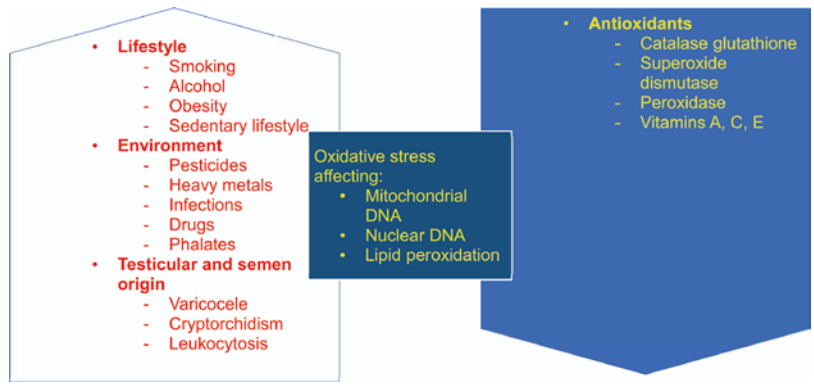

Fig. 32.1 Oxidative stress model. (Based on data from Ref. [21])

heterogeneous, and inadequate to deny or confirm this causation.

Undoubtedly, varicocele and male infertility are linked. It might be a clear association, or it might be that varicocele can cause a detrimental effect on a specific subgroup of infertile men. The presence of a varicocele should not be considered an all-or-none etiology. Instead, varicocele might lead to a spectrum of clinical manifestations. In some cases, varicocele is considered coincidental to male factor infertility, in other words, a direct cause. These scenarios depend mainly on the individual's genetic and epigenetic compositions and susceptibilities.

Markers, such as proteomics, inhibin B, and ORS, are providing a new horizon into individualizing care of infertile patients with varicocele. Advancements have been made towards developing specific biomarkers that might give the caregivers an insight of infertility etiology and direct the therapeutic interventions and follow-up.

Further well-controlled research and randomized studies should be dedicated to this field in order to validate the current evidence. Particular attention should be given to studying the exact effect of varicocelectomy on the different fertility prognostic factors and developing cost-effective and clinically available markers for patients with varicocele in order to better understand the extent of its potential pathophysiological injury.

Review Criteria

Search engines such as PubMed, Medline, and ScienceDirect were used for an extensive search looking at scientific evidence, papers, and studies regarding the debatable effect of varicocele on male infertility. The search included reports between the years 1950 till 2017. English and non-English studies were included. The keywords used were mainly "varicocele," "male factor infertility," "varicocele induced infertility," "pregnancy outcomes," "varicocele epidemiology," "varicocele pathophysiology," "proteomic markers," "sperm apoptosis," "varicocelectomy," "varicocele-induced oxidative stress," and "sperm DNA integrity."

Multiple Choice Questions and Answers

1. Regarding fertility in men with varicocele, most of the studies reported:
   (a) A minority of men with varicocele are fertile.
   (b) Varicocele is very infrequent in the general population, less than 1%.
   (c) Varicocele's physical exam is not examiner dependent.
   (d) **More than half the men with varicocele are fertile.**
2. Regarding varicocele and semen parameters:
   (a) Semen parameters were shown to be specific and sensitive in predicting varicocele presence.
   (b) **Studies have shown that abnormal semen parameters are found in infertile men regardless of varicocele presence.**
   (c) "Stress pattern" semen analysis associated with varicocele translates only into isolated oligospermia.
   (d) All men with varicocele have abnormal semen analysis.
3. Regarding varicocelectomy effect on pregnancy rates and semen analysis:
   (a) Varicocelectomy showed universal improvement in pregnancy rates.
   (b) **The Cochrane reviews regarding varicocelectomy and pregnancy rates had rather low evidence.**
   (c) Semen analysis after a varicocelectomy showed improvement in all the studies.
   (d) Most of the studies regarding semen analysis after varicocelectomy are well randomized and controlled.
4. Regarding proteomic markers:
   (a) Specific biomarkers have not been identified in patients with varicocele.
   (b) **Can be utilized in male fertility as a diagnostic tool, prognostic factor, and treatment success indicator.**
   (c) Are currently cost-efficient and used in most clinical settings.
   (d) Are easy to identify and reproduce since they are not technically challenging.

5. Regarding inhibin B:
   (a) It is produced by Leydig cells.
   (b) **A normal level of inhibin B in patients with varicocele might indeed point towards a normal spermatogenesis and a subsequent intact fertility.**
   (c) It has a feedback loop on LH secretion.
   (d) Levels always improved after varicocelectomy.

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Is There a Role for Testicular Biopsy in Men with Varicocele?

33

David Guo, Ali Amin, and Kathleen Hwang

Key Points

- Varicocele repair in men with nonobstructive azoospermia (NOA) may result in the appearance of sperm in the ejaculate, improved surgical sperm retrieval, and higher clinical pregnancy rates.
- Testicular biopsy prior to varicocele repair can demonstrate three main testicular histopathologies: hypospermatogenesis (HS), maturation arrest (MA), and Sertoli cell-only (SCO) pattern. HS and MA are associated with a more favorable outcome following varicocele repair.
- The clinical relevance of testicular biopsy is controversial because it may not represent the entire testis and its technique and interpretation are variable, implying that it may not ultimately change clinical management.
- Nevertheless, the potential for testicular biopsy to provide prognostic information should be discussed with the patient with NOA who is considering varicocele repair, and biopsy should be entertained if it will affect the patient's clinical course.

Introduction

Approximately 1% of all men and 15% of men who present with infertility are found with azoospermia, which is defined as the absence of sperm, confirmed by examination of two centrifuged samples [1]. Azoospermia may be classified as obstructive or nonobstructive. Obstructive azoospermia suggests a blockage in the natural exit of the sperm, whether in the epididymis, vas deferens, ejaculatory ducts, or urethra. Nonobstructive azoospermia indicates a problem with the production of sperm itself, due to some level to testicular failure. There are numerous ways to categorize testicular failure: acquired versus congenital, and irreversible versus potentially reversible.

Approximately 5% of men who are evaluated for infertility present with both varicocele and nonobstructive azoospermia [2]. Because varicoceles represent a potentially reversible cause of

D. Guo (✉)
Department of Urology, Rhode Island Hospital/Brown University, Providence, RI, USA

A. Amin
Department of Pathology and Laboratory Medicine, Rhode Island and the Miriam Hospitals/Brown University, Providence, RI, USA

K. Hwang
Department of Urology, Rhode Island Hospital/Brown University, Providence, RI, USA

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spermatogenic dysfunction, increased attention has been directed toward the outcomes of varicocele repair in men with nonobstructive azoospermia. While the American Urological Association and American Society for Reproductive Medicine recommend offering varicocele repair for infertile men with a palpable varicocele and an abnormal semen parameter [3], the utility of varicocele repair in men with nonobstructive azoospermia, the most severe seminal derangement, is less established. Nevertheless, various studies have suggested that 21% to 55% of men with nonobstructive azoospermia and a clinical varicocele have sperm in their ejaculate after varicocele repair [2].

Because not all men with nonobstructive azoospermia will benefit from varicocele repair, studies have investigated the factors that may predict a favorable outcome. Characteristics including patient age, infertility duration, varicocele grade and laterality, FSH level, and testicular volume do not appear to be correlated with sperm recovery [4]. However, testicular histopathology has been shown in multiple studies to be predictive of the outcome [4, 5]. For this reason, testicular biopsy has been advocated with the hope of identifying the subset of men with both nonobstructive azoospermia and varicocele who are most likely to benefit from varicocele repair.

Testicular Biopsy: Rationale and Technique

The main rationale for testicular biopsy is to avoid unnecessary surgery in the operating room. In addition, this may avoid a potential delay in selecting the correct treatment, such as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), which may be especially important for couples who are facing a time-limited fertility window. While the goal of testicular biopsy is to provide sufficient tissue to allow for histopathologic diagnosis, the sperm recovered via testicular biopsy may also be used immediately or cryopreserved for use in IVF/ICSI. In this way, testicular biopsy may serve both a diagnostic and therapeutic purpose.

There are a variety of methods of testicular biopsy, including percutaneous testicular aspiration, percutaneous testicular biopsy, and open testicular biopsy [6]. These methods may be performed in the office setting using local anesthetic with a spermatic cord block if tolerated by the patient. They may also be performed in the operating room using spinal anesthesia, monitored anesthesia care or general anesthesia. Percutaneous fine-needle aspiration (FNA) with a 23-gauge needle may obtain sperm for use in ICSI, but will generally not preserve the architecture of the tubules needed for histological evaluation. Percutaneous biopsy utilizes a tru-cut needle (e.g., 16 or 18 gauge), which provides a better tissue sample. Open testicular biopsy, which may be augmented by loupes or the microscope, involves making a small 2–3 millimeter incision in the scrotal skin down to the tunica albuginea to liberate a small sample of seminiferous tubules, which are excised and sent for histopathologic interpretation.

Testicular Biopsy: Histopathology

Testicular biopsy can provide sperm for use in ICSI and also provide histological information. Histologic analysis is performed with light microscopy and characterizes the presence of germ cells versus Sertoli cells, the amount of spermatogenesis, and the most advanced stage of sperm development [7]. At the most primitive and immature stem cell stage is spermatogonia, which divide via mitosis into primary (diploid) spermatocytes and then via meiosis I into secondary spermatocytes (haploid) and via meiosis II into spermatids prior to becoming spermatozoa (Fig. 33.1).

The normal histological sample of the testis will demonstrate seminiferous tubules with abundant germ cells in all stages of spermatogenesis (Fig. 33.2). Testicular histopathologies such as hypospermatogenesis, early- and late-stage maturation arrest, and Sertoli cell-only represent a deviation from this normal process (Table 33.1). In hypospermatogenesis (HS), there are germ cells in all stages of spermatogenesis, but not in abundance. In maturation arrest (MA), there are germ cells in multiple stages of spermatogenesis,

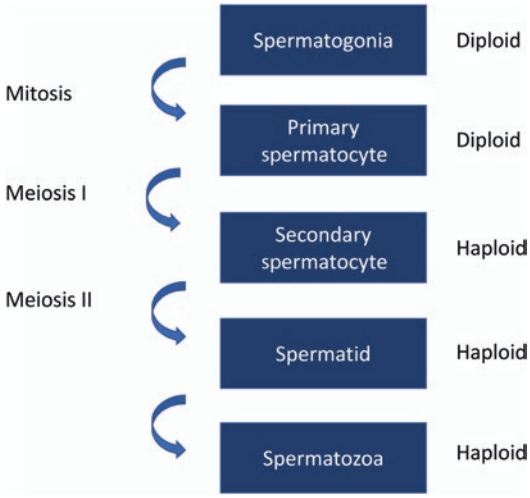

Fig. 33.1 Stages of spermatogenesis. The production of haploid sperm (spermatozoa) via spermatogenesis proceeds through multiple stages of division, starting from the diploid spermatogonial stem cells

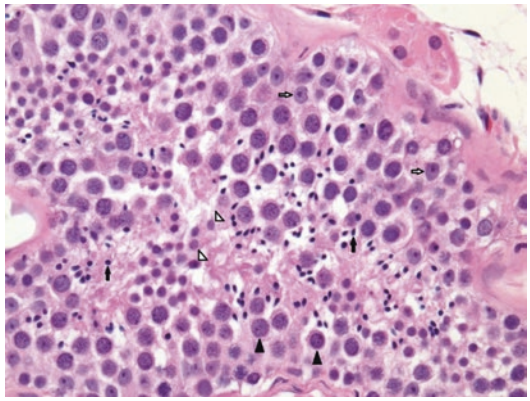

Fig. 33.2 Seminiferous tubule with normal spermatogenesis. (Note that the tubule is populated with ample germ cell lineage. Sertoli cell nuclei are mainly seen at the periphery (white arrows) with many primary spermatocytes showing course chromatin (black arrowheads), secondary spermatocytes (white arrowheads), and abundant spermatozoa in the midst of the tubule (black arrows) (H&E, 400X))

but no complete mature sperm. This is further distinguished as early maturation arrest (at the spermatocyte stage) and late maturation arrest (spermatid stage) [8]. In Sertoli cell-only (SCO), there is an absence of germ cells and only Sertoli cells are observed within the seminiferous tubule architecture (Fig. 33.3).

Table 33.1 Testicular histopathologic patterns

Histopathologic pattern	Characteristic
Hypospermatogenesis	Germ cells at all stages of development, with decreased number
Maturation arrest	*Early*: germ cells at stages of development reaching spermatocyte stage
	Late: germ cells at stages of development reaching spermatid stage
Sertoli cell-only	Absence of germ cells within seminiferous tubules

Testicular Histopathology and Varicocele Outcomes

Multiple studies of men with nonobstructive azoospermia receiving testicular biopsy before or at the time of varicocele repair have suggested that the discovery of HS and MA on testicular biopsy is more likely to show improved semen parameters than SCO, although SCO does not automatically portend the absence of sperm. Three meta-analyses of the existing literature have recapitulated these findings [9–11].

Presence of Sperm in Ejaculate

Each meta-analysis evaluated the relationship between testicular histopathology and the presence of sperm in the post-varicocele repair ejaculate (Table 33.2).

The 2010 meta-analysis by Weedin and colleagues included 8 studies and 233 men with nonobstructive azoospermia who underwent varicocele repair (microscopically or by embolization) and defined a successful outcome as either the discovery of sperm in the ejaculate or spontaneous pregnancy. [9] In aggregate, 39.1% had sperm in the ejaculate and 6% achieved spontaneous pregnancy. Success rates were found to be 54.5% with HS, 42.1% with MA, and 11.3% with SCO. This study further distinguished between early and late MA; 45.8% of patients with late MA had a successful outcome, but none of the men with early MA had a successful outcome.

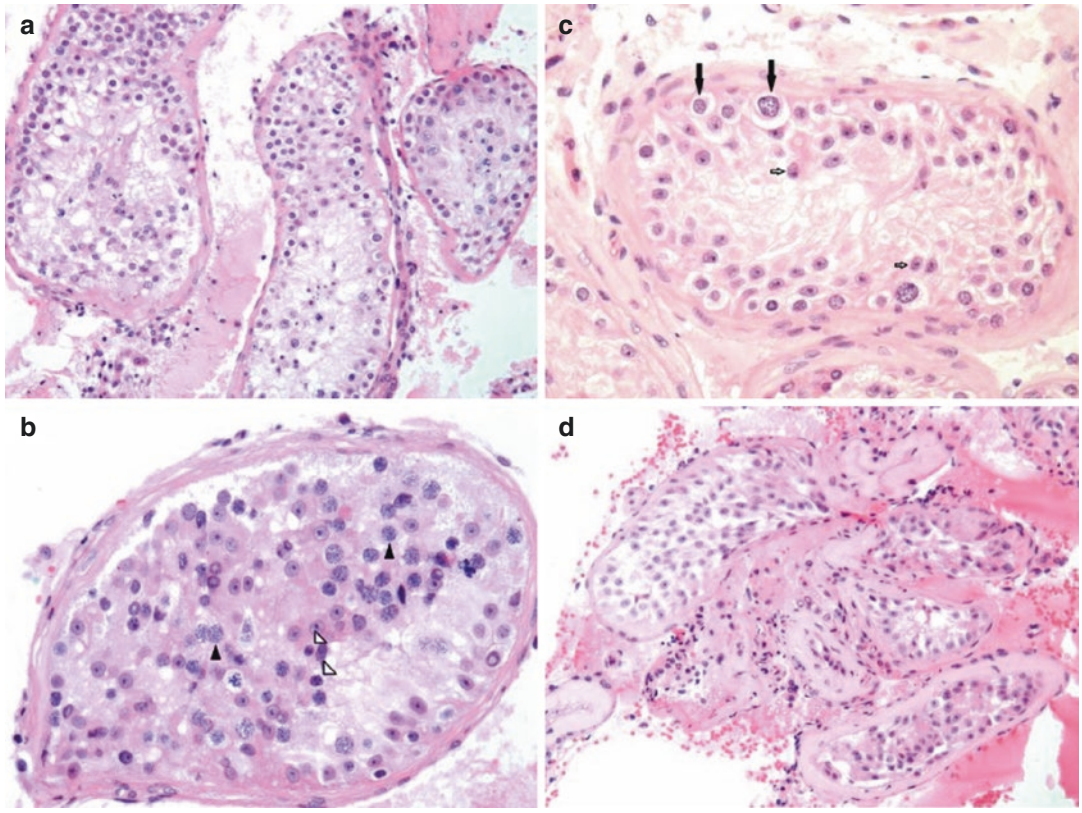

Fig. 33.3 Representative histology from testicular biopsies. (**a**) Hypospermatogenesis. Note presence of all germ cell lineages (including spermatids and spermatozoa) but in reduced number (H&E, 200X). (**b**) Early maturation arrest (spermatocytic arrest). Note scattered spermatogonia at the periphery of seminiferous tubules (dark arrow) with some degenerative atypia among Sertoli cells (light arrow). No evidence of more mature germ cells is present (H&E, 400X). (**c**) Late maturation arrest (spermatocytic arrest). Note presence of abundant number of primary spermatocytes (dark arrowhead) and scattered secondary spermatocytes (light arrowhead). No evidence of spermatozoon is identified (H&E, 400X). (**d**) Sertoli cell-only pattern (germ cell aplasia). Note complete absence of germinal layer with abundant Sertoli cells. Also, there is peritubular fibrosis marking the chronicity of the disease (H&E, 200X)

The 2014 meta-analysis by Elzanaty evaluated 5 studies comprising 90 men with nonobstructive azoospermia who underwent either macroscopic or microscopic varicocele repair [10]. This meta-analysis investigated the return of sperm in the ejaculate, finding these rates to be 60.0% with HS, 46% with MA, and 3.0% with SCO. This study also distinguished between early and late MA, finding that 50% (2/4) of men with late MA and none (0/3) of the men with early MA had sperm in the ejaculate, but noted that this difference was not statistically significant ($p > 0.05$).

The 2016 meta-analysis by Esteves and colleagues examined the outcomes of microscopic varicocele repair for men with nonobstructive azoospermia who were confirmed histologically with testicular biopsy either before or at the time of repair [11]. This encompassed eight studies comprising 161 men and demonstrated the presence of sperm in the ejaculate in 56.2% of men with HS, 35.3% of men with MA, and 9.7% of men with SCO.

Surgical Sperm Retrieval Rate

Another outcome that has been examined is the surgical sperm retrieval rate after varicocele repair. Only one study has examined this outcome in relation to testicular histopathology [12].

Table 33.2 Presence of sperm in ejaculate according to testicular histopathology

Meta-analysis	Studies and population size	Type of varicocele repair	HS	MA	SCO
Weedin et al. (2010) [9]	8 studies, *n* = 233	Microscopic and embolization	54.5% (30/55)	42.1% (24/57)	11.4% (5/44)
Elzanaty (2014) [10]	5 studies, *n* = 90	Macroscopic and microscopic	60.0% (18/30)	46.2% (12/26)	2.9% (1/34)
Esteves et al. (2016) [11]	8 studies, *n* = 161	Microscopic	56.2% (27/48)	35.3% (18/51)	9.7% (6/62)

HS hypospermatogenesis, *MA* maturation arrest, *SCO* Sertoli cell-only

Of 17 men with nonobstructive azoospermia who underwent varicocele repair, 9 (53%) remained azoospermic after repair and subsequently underwent microsurgical testicular sperm extraction (microTESE). Sperm was found in four (44.4%) of the men who underwent microTESE: 1/1 (100%) had HS, 2/2 (100%) had MA, and 1/6 (16.7%) had SCO pattern.

Limitations of Testicular Biopsy

There are several limitations regarding the predictive ability of testicular histopathology. First, the technique and interpretation of testicular biopsy in the aforementioned studies are not standardized. Some may utilize core needles, while others use open excisional biopsy, with or without loupe or microscopic assistance. The number of seminiferous tubules examined is also variable. Furthermore, the histologic interpretation of a testicular biopsy may differ significantly among pathologists. One study has shown that discrepant interpretations between pathologists would have resulted in 27% of cases having a different clinical management course [13].

Moreover, a major limitation of testicular biopsy is that the heterogeneous composition of the testis implies that a single biopsy may not be representative of the entire testis. In an "FNA mapping" study of the testis, Turek and colleagues found that a third of patients who had no sperm on initial testis biopsy were found with foci of spermatogenesis during systematic FNA at separate sites [14]. Ramasamy and colleagues evaluated men who underwent microTESE after failing to find sperm on biopsy; 51% of men with 1–2 prior biopsies and 23% of men with 3–4 biopsies still had sperm found on microTESE [15]. In addition, a unilateral testis biopsy may not be indicative of the histology of both testes. Plas and colleagues reviewed testis biopsy samples in 100 azoospermic men and found that 28% had differences in histopathology between the two testes [16].

Another consideration is the recent finding that histopathology may change after varicocele repair. Ustuner and colleagues performed testicular biopsies at the time of varicocele repair, followed by microTESE [17]. Of the 14 patients who had SCO on testicular biopsy, 6 (42.9%) of these patients had either maturation arrest or foci of spermatogenesis identified at subsequent microTESE. Therefore, an initial finding of unfavorable SCO pathology does not rule out the possibility of a future change to a more favorable histopathology.

Though rare, there are certain risks associated with testicular biopsy. These include hematoma, local inflammation, and infection [18]. Injuries to the testicular artery may lead to atrophy of the testis and hypogonadism, and disruption of the epididymis may cause scarring and obstruction [7]. In addition, testicular biopsy may result in the removal of the few rare foci of spermatogenesis in an otherwise barren testis, further limiting reproductive potential [19].

It is worth noting that testicular biopsy has historically been investigated in another group for whom the prognosis of varicocele repair is particularly important: adolescents. A study of 13 adolescents with varicoceles revealed certain histopathologic changes; nine had tubular sclerosis and six had small vessel sclerosis [20]. However, there appears to be little predictive

value of these biopsies. Hadziselimovic and colleagues followed 25 adolescent patients who underwent bilateral testis biopsy and varicocele repair and found no correlation between testis histology and semen parameters at 10-year follow-up [21]. Given the invasive nature of biopsy, testicular biopsy is not widely performed in adolescents.

New Directions

Interpretation and histopathologic analysis of testicular biopsy samples vary among pathologists. Management decision based on histopathology is doubtful at the moment [13]. In an effort to provide more predictive information based on testicular biopsies, Shiraishi and colleagues have examined the genome-wide mRNA expression profiles of testicular tissue in men with MA who have undergone varicocele repair [22]. Importantly, they discovered that upregulation of a cell-cycle-related gene expression in men with MA histopathology was associated with sperm recovery. This is a significant step in the use of modern genomic technology to improve prognostic testing in men with nonobstructive azoospermia and varicoceles. However, given that this still requires an invasive testicular biopsy, Clavijo and colleagues have noted that further scientific inquiry should be directed toward the development of noninvasive, blood-based prognostic testing that may determine the best candidates for varicocele repair [23].

Conclusion

The current published literature indicates that testicular biopsy does provide prognostic information. In patients with nonobstructive azoospermia, repair of a clinical varicocele is associated with improved prognosis in the presence of sperm in the ejaculate for men with hypospermatogenesis and maturation arrest. The outcomes in relation to surgical sperm retrieval, clinical pregnancy, and live birth rates are limited.

Nevertheless, it is important to highlight that even with a diagnosis of SCO on testicular biopsy, up to 11% of men had sperm found in their post-varicocele repair ejaculate [11] and nearly 17% had surgically retrieved sperm [12]. While it would be reasonable to forego varicocele repair if no men with SCO experienced improvement of seminal parameters, failing to offer varicocele repair for men with SCO in light of these results may prematurely deprive them of a potentially beneficial fertility intervention [24].

In practice, the relative benefit of testicular biopsy depends on the specific goals of the couple. If the couple would proceed with intervention despite prognostic probabilities, then testicular biopsy would be unnecessary. It is therefore important to counsel patients carefully according to the published literature. Testicular biopsy does provide more information to counsel patients, but ultimately, if the management course would not change (i.e., the patient who desires biological paternity is determined to undergo intervention no matter the probability of success), testicular biopsy would not be a necessary step prior to varicocele repair.

Review Criteria

An extensive query was performed of studies investigating the role of testicular biopsy in the management of males with varicocele using the search engines PubMed and Google Scholar. The start and end dates for the searches were April 2018 and August 2018, respectively. The overall strategy for study identification and data extraction was based on the following keywords: "varicocele," "azoospermia," "testicular biopsy," "testicular histopathology," "infertility," and "semen parameters." Articles published in languages other than English were not considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included. Websites and book chapter citations provide conceptual content only.

Multiple Choice Questions and Answers

1. Testicular biopsy has been proposed as a potentially useful adjunctive procedure in infertile men with which of the following conditions?
   (a) Clinical varicocele and obstructive azoospermia
   (b) Subclinical varicocele and obstructive azoospermia
   (c) **Clinical varicocele and nonobstructive azoospermia**
   (d) Subclinical varicocele and nonobstructive azoospermia
2. Recovery of sperm in men with nonobstructive azoospermia after varicocele repair does not appear to be related to the following factors, *except*:
   (a) Patient age
   (b) Varicocele grade
   (c) **Testicular histology**
   (d) Testicular volume
3. Which testicular histology is associated with the least favorable prognosis for the presence of sperm following varicocele repair in men with nonobstructive azoospermia?
   (a) Normal histology
   (b) Maturation arrest
   (c) Hypospermatogenesis
   (d) **Sertoli cell-only**
4. Each of the following statements is considered a limitation of using testicular biopsy to determine prognosis following varicocele repair, *except*:
   (a) Histologic interpretation of testis samples may vary considerably between individual pathologists.
   (b) **Studies of testicular histology have failed to demonstrate any differences in outcome after varicocele repair.**
   (c) The heterogeneous composition of the testis implies that a single biopsy may not be representative of the entire testis.
   (d) Testis histology may evolve following varicocele repair.
5. Which outcome has been mostly frequently investigated in relation to the role of testicular histology and varicocele repair in men with nonobstructive azoospermia?
   (a) **Presence of sperm in ejaculate**
   (b) Surgical sperm retrieval rate
   (c) Clinical pregnancy rate
   (d) Live birth rate

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34 Should Bilateral Varicocele Repair Be Recommended in Cases of a Clinical Varicocele and Contralateral Subclinical Varicocele?

Juan D. Cedeno and Alan Scott Polackwich Jr.

Key Points

- Subclinical varicocele is common in the general population, most of whom have no issues with conception.
- Some studies suggest that subclinical varicocele may progress to clinical varicocele (explaining secondary infertility in these patients), but this is in the minority of patients.
- Subclinical varicocele likely has an effect on semen parameters, but less than clinical varicocele.
- In patients with both clinical and subclinical varicocele, bilateral ligation leads to increase in seminal parameters, but in most studies, no more than unilateral ligation.
- Pregnancy rates likely improve with ligation of subclinical varicoceles over observation, but not as much as patients with clinical varicoceles.

Introduction

Varicocele is a clinical entity commonly seen by the urologist and male fertility specialist. It has been estimated that the prevalence of varicocele is 15–20% in the general population and 30–40% in the infertile population [1–3]. Categorization of varicoceles is based on Dubin and Amelar's system grading from I to III [4]. Varicocele is characterized by the distension of the pampiniform plexus within the scrotum, which occurs secondary to the retrograde flow via spermatic vein due to incompetent or absent valves, normally diagnosed by physical examination with the patient in upright position and during Valsalva. However, when retrograde blood flow occurs, and the scrotal venous plexus is not palpably distended, the varicocele will not be apparent clinically and can be identified only with radiographic techniques, such as ultrasound, color Doppler imaging, or venography. This is considered a subclinical varicocele. This condition is found in 44% of fertile men and in 60% of those visiting infertility clinics, sometimes associated with a clinical varicocele [5]. Due to the prevalence of this condition, it is unclear how it affects fertility and controversial as to whether it requires treatment. In this chapter, we will focus on the subclinical varicocele: how subclinical varicocele may affect infertility, importance of treatment, and the outcomes of surgery [2–4].

J. D. Cedeno
Department of Urology, Mount Sinai Medical Center, Miami Beach, FL, USA

A. S. Polackwich Jr. (✉)
Columbia Department of Urology at Mount Sinai Medical Center in Miami Beach, Mount Sinai Medical Center, Miami Beach, FL, USA

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Diagnostic Methods

Multiple methods of diagnosis have been used to identify varicoceles in general, typically relying on physical exam. Subclinical varicoceles are those whose veins in the pampiniform plexus are not palpable by physical examination but are able to be identified with retrograde blood flow during Valsalva. For this reason, there are a variety of diagnostic tools used in the literature, though overwhelmingly, the most commonly used imaging technique is the color Doppler ultrasound.

One of the oldest imaging techniques, venography was introduced in 1966, visualizing retrograde flow in the internal spermatic vein. Spermatic venography had long been the gold standard for diagnosis of varicocele [6], generally considered the most sensitive test, as nearly 100% of clinical varicoceles demonstrate spermatic vein reflux [7]. By contrast, physical examination detects only 71% of the varicoceles evident on venography [7, 8]. In addition to being diagnostic, it can be therapeutic as well, with the use of sclerotic or occlusive methods [7, 9].

Nuclear scans have also been used to diagnose varicoceles, especially those not obvious on physical exam such as subclinical varicocele, though this is now rarely used. Although it is a sensitive test, it can be fraught with difficulties due to issues in technique and patients with bilateral lesions. In this test, 15 mg of stannous pyrophosphate is injected IV to tag a pool of red blood cell (RBC). Patients are placed in the supine position with midline scrotal raphe marked. After a 20-min delay, 20 mCi of 99 m TC pertechnetate diffuses into the red blood cell and becomes reduced by the intracellular stannous ions, preventing elution from the red blood cells. The radionuclide scan is interpreted as positive if any combination of one or all of the following is present: asymmetry of activity on the flow study, static images with a small area of focal activity in either hemiscrotum, or a difference of over 10% on the computerized count ratios when right and left sides were compared. Due to the manner in which this is performed, determining bilateral varicoceles or severity of varicocele is difficult, especially in the case of subclinical varicoceles. In one study comparing ultrasound to nuclear scan, sonography was positive for subclinical varicocele in 95% patients, whereas nuclear scanning was considered positive in only 55% [10]. Due to the widespread use of other imaging techniques that are easily accessible to the urologist, this is rarely used [10].

Duplex ultrasound of the testes has become the standard method for assessing the diameter of the internal spermatic veins and the presence of reflux during Valsalva maneuver. Due to a number of ultrasounds performed during the evaluation of the infertile male, it is on ultrasound that the majority of subclinical varicoceles are being detected. Currently, a diameter of 3 mm has been recognized as the limit above which a varicocele could become palpable, though some studies utilize 4 mm or even 2 mm [11–13]. Clinical research has demonstrated that left clinical varicocele is commonly accompanied by a lesser-grade or subclinical right-sided varicocele [11, 14]. In one study of 112 men with clinical varicocele, 20 of them had subclinical varicocele as well [15].

How Subclinical Varicocele Affects Fertility

With advancement in imaging techniques, we have been able to detect more subclinical varicoceles. Whether or not they require repair is a matter of discussion. First, it is important to determine whether they are even clinically relevant. In patients having both a clinical and subclinical varicocele, it is difficult to determine the impact of the subclinical varicocele. Thus, we turn to studies evaluating subclinical varicoceles in isolation.

In a paper from 2016, Hallak et al. looked at the Androscience database from 2001 and 2015

and located 128 men with bilateral unrepaired subclinical varicoceles, 46 of which participated in the study. Initial median sperm concentration was 38.22 million/ml, total motility 60.2%, progressive motility 37%, and WHO normal morphology (third edition 1992) 26%. Creatine kinase activity as an indicator of sperm quality and maturity measurement was also measured and was 0.107 ± 0.086 IU 10–8 sperm (normal <0.036 IU 10–8 sperm). These data suggested some sperm dysfunction at baseline. Of the initial 46 patients, 27 were followed for over 5 years, total motility decreased to 44% and morphology to 21%, and CK activity increased to 0.221 ± 0.116 IU 10–8 sperm. While the effect is not definitive, it does suggest that subclinical varicoceles may impair fertility, though whether clinically significant is questionable [16].

Some practitioners may argue subclinical varicoceles develop into clinical varicoceles and therefore should be repaired when the opportunity presents itself because even very small varicoceles or subclinical varicoceles should be identified and treated due to a progressive rather than static effect upon male infertility, suggesting that the presence of subclinical varicocele may result in some abnormalities in semen analysis and subsequently infertility [2]. It has been shown that a portion of subclinical varicoceles may progress rather than spontaneously resolve. In a study of children with subclinical varicocele, 28% progressed to a clinical varicocele (95% CI 14% to 15%) during a 4-year period [17, 18]. It is that progressive effect from subclinical varicocele to clinical varicocele that may result in subsequent seminal damage and male infertility seen in other studies. In 1993, Witt et al. conducted a study where 2989 patients were evaluated for infertility, and 128 out of 255 (50%) and 177 out of 255 (67%) were diagnosed with primary and secondary infertility related to varicocele demonstrating loss of fertility in previously fertile patients, suggesting that varicocele is a progressive rather than static lesion [19].

There may be an effect on testicular size, though studies are conflicting. One study by Zini et al. demonstrated, with the use of ultrasound, that in men with left subclinical varicocele, left testicular volume was significantly lower when compared to contralateral unaffected testes (13.2 vs 14.7 ml, $p < 0.001$) [18]. Another study found no significant difference in testicular volume in infertile patients with subclinical varicocele and infertile patients without subclinical varicocele [19]. Though it may be that only a subset of these patients have significant issues, it was found that patients with subclinical varicocele and peak retrograde flow (PRF) >29 cm/s with total testicular volume <27 cc have higher chance of male subfertility [2].

In a study from 2010 of patients with clinical varicocele, elevation of scrotal temperature was found to be one of the major factors that impaired spermatogenesis and steroidogenesis in testes with varicocele. This was closely associated with oxidative stress, following the apoptosis of germ cells [20]. A study of 150 men with subclinical varicocele matched with men without subclinical varicocele found that subfertile patients (more than one abnormal semen parameters: count <20× 10^6/ ml, motility <50%, or abnormal morphology <30% by two subset of analysis) with subclinical varicocele had slightly higher scrotal temperature (34.9 °C) than fertile patients with subclinical varicocele (34.4 °C) and normal control (34.44 °C) [21].

In recent years, there has been a growing interest in sperm DNA fragmentation, now linked to longer conception time and higher miscarriage rates. High percentages of fragmented DNA are found in the sperm of patients with varicoceles. A study of 60 infertile men with varicocele classified them into four different cohorts: (1) 15 males with non-treated grade I clinical varicocele (CV), (2) 16 males with subclinical varicocele diagnosed by scrotal Doppler ultrasound (SCV), (3) 19 surgically treated clinical varicocele (T-CV), and (4) 10 surgically treated subclinical varicocele (T-SCV). All of the sperm samples were processed with multiple DNA fragmentation tests

(terminal transferase *dUTP nick end labeling [TUNEL] assays, sperm chromatin dispersion test [SDC], sperm chromatin structure assays*). Patients with T-CV showed significant lower sperm DNA fragmentation compared to untreated in both SCD and SCSA assays ($p < 0.05$). However, no benefits were found on T-SCV in any of the methods [21], though the technique of ligation used was not a subinguinal technique, considered the gold standard, and numbers of the study were quite low [22].

Effects of Repair of Subclinical Varicocele

Sperm Concentration

While the data suggest that subclinical varicocele may affect fertility at least in some patient populations, it is less clear whether the risk of surgery outweighs the benefits from ligation. One prospective study compared patients with left clinical and right subclinical varicocele who were randomly assigned to two groups: bilateral varicocelectomy (BV, $n = 51$) and unilateral left varicocelectomy (LV, $n = 53$). The diagnosis of subclinical varicocele was made by Doppler ultrasound defined as the presence of internal spermatic vein >3 mm during Valsalva and not seen during physical examination in a warm room and patient in supine position. Varicocelectomy was done by retroperitoneal approach. Semen analysis was done two times preoperatively and 12 months after surgery. Postoperatively no significant differences were found in sperm concentration between the two groups, but there was a significant increase in sperm concentration in general (left varicocele = 7.6 ± 2.3 pre-op vs 24.4 ± 10.3 post-op vs bilateral varicocele = 7.1 ± 2.1 pre-op vs 23.7 ± 11.1 post-op) ($p < 0.05$) [12]. Similar results were found by Jarow et al. in 1996. They found that repair of SCV did not significantly improve semen quality compared to larger, clinical apparent varicoceles. In fact, in this study, they found that an equal number of patient's seminal counts worsen than improved [23].

More recently, a meta-analysis of 548 patients, 276 who underwent subclinical varicocelectomy and 272 who had no surgical intervention or only pharmacological intervention with clomiphene, demonstrated no statistically significant difference between groups (mean difference [MD] 0.92, 95% CI −0.36 to 0.219) [24]. A subsequent prospective, single-institution randomized controlled trial (RCT) with 358 patients evaluated the efficacy of bilateral varicocelectomy ($n = 179$) vs unilateral varicocelectomy ($n = 179$) in those with left varicocele and right subclinical varicocele. They utilized a microscopic subinguinal approach. Preoperatively, both groups had comparable seminal characteristics. Semen analysis was done every 3 months with a mandatory follow-up at 12 months with semen analysis. The improvement in sperm concentration was statistically significant ($p < 0.05$). Sperm concentration improved more in the bilateral group by 19×10^6/ml versus 11.1×10^{6E} ml ($p = 0.041$) in the unilateral group. The authors conclude that bilateral repair in patients with left varicocele and right subclinical varicocele resulted in greater improvement in seminal parameters and improvement in pregnancy rate [25]. Though some of the data is conflicting, there is increasing evidence that a bilateral varicocelectomy may be a superior treatment in patients with a clinical and subclinical varicocele.

Sperm Motility

Other studies have also investigated the effect of the repair of a subclinical varicocele on motility. One single-center RCT compared patients with a clinical varicocele to those patients with a subclinical varicocele. A total of 142 patients were enrolled. At the time of enrollment, a semen analysis was obtained and reobtained at 3 and 6 months after surgery. All the patients included in the study underwent high ligation of internal spermatic vein. Subclinical varicocele was identified by scrotal US in patients with three dilated veins, at least only one with diameter >3 mm. While there was a significant improvement in motility and count in the clinical varicocele group

(for varicocele grade II the percentage of motile sperm pre-op 46.25 ± 20.75 vs post-op 66.4 ± 20.2, $p < 0.05$ and for varicocele patient with grade III pre-op 42.6 ± 15.4 vs post-op 78.55 ± 13.85, $p < 0.05$), there was no significant improvement in motility in the subclinical group (60.7 ± 12.1 pre-op vs 65.4 ± 9.8 post-op, $p < 0.05$) [26].

Another study of 145 infertile patients with left clinical varicocele and right subclinical varicocele also evaluated the effect of varicocelectomy on motility. Doppler ultrasound was used for diagnosis, and varicocele was defined when retrograde flow was noted in a vessel >2 mm in diameter during Valsalva. The patients were then randomized to either bilateral ($n = 73$) or unilateral ($n = 72$) inguinal (non-microsurgical) varicocelectomy. After surgery, semen analysis was obtained every 3 months in the first year and biannual thereafter. The majority of patients in both groups had a clinical varicocele grade II or grade III (83.5% and 87.5%). After surgery, significant improvements in sperm concentration, sperm motility, progressive motility percentage, and percentage of sperm with normal morphology were seen in both groups. This further suggests that while varicocele repair is helpful for patients, treatment of a subclinical varicocele may not be needed [27].

Another retrospective study from a single-center university infertility clinic assessed fertility after varicocelectomy in 143 men with subclinical varicocele. Inclusion criteria include no additional clinical factors associated with infertility for a period of >1 year and a normal hormonal profile, a normal testicular size, no prior treatment for infertility, and a subclinical left-sided varicocele. Varicocele was diagnosed by physical exam and Doppler ultrasound in the supine and standing position before and during the Valsalva maneuver, whereas subclinical varicocele was defined as presence of veins with a maximal diameter of >3 mm on an ultrasound during the Valsalva maneuver not palpable or noticed during physical examination in a warm room and patient standing up. Patients were able to determine the treatment option: microsurgical varicocelectomy ($n = 25$) or medical treatment with L carnitine (3 gr/ day/po x 6 months) ($n = 93$) or no intervention ($n = 25$). Semen analysis was performed twice before treatment and reevaluated twice 6 months after treatment. Pregnancy rates were estimated 1 to 2 years after treatment by telephone survey. Preoperatively there was no significant difference in semen parameters, the patients' or partners' age, or the size of testes among the three groups. The group who elected surgery had statistically significant improvement in semen concentration from 39.3 ± 36.0 million/ml to 57.5 ± 46.9 million/ml ($p = 0.005$); however, there were no improvements in the other parameters. None of the other groups had any improvement in seminal parameters. This study demonstrated that surgical treatment of subclinical varicocele in infertile men without a clinical varicocele resulted in improved seminal concentration (though unclear if clinically significant) and no significant improvement in sperm motility or morphology. Interestingly, there was an improvement in pregnancy rates, surgery (60%), medication (34.5%), and observation (18.7%) ($p = 0.031$), though this study had a small surgery group compared to the medical group, no randomization, and a high rate of patients who were lost to follow-up (36%) [5].

Another study by Cantoro et al. evaluated 337 men from 18 to 37 years old diagnosed with left-sided varicocele and subclinical varicocele diagnosed by Doppler ultrasound. Two hundred eighteen patients underwent retrograde embolization of the left internal spermatic vein (ISV), whereas 119 who did not undergo any intervention or medical treatment were included as the observation group. The mean of three-semen analysis was evaluated at baseline and 6 months after treatment. Semen analysis after 6 months showed significant improvement in sperm concentration and total motility in the intervention group: sperm concentration (M/ml) was 37.4 ± 10.7 vs 17.5 ± 5.6 ($p < 0.05$) and total sperm motility was (%) 46.6 ± 9.5 vs 31.5 ± − 9.1, $p < 0.05$. No improvement was seen in LH, FSH, and testosterone. The percutaneous embolization of the ISV in infertile patients with one or more abnormal semen parameters is effective in improving pregnancy rate and semen [3]. Caveats

in these studies include the following: the WHO reference range used was the 1999 version not the 2010, and randomization was done based on patient selection [22].

Lastly, in a recent systematic review and meta-analysis, which included 13 studies with 1357 patients comparing corrected subclinical varicocele vs control groups (no intervention), and corrected subclinical varicocele versus varicocelectomy in clinical varicocele, it was found that subclinical varicocele undergoing varicocelectomy resulted in a mean improvement of 9.95 (95% CI 5.41–14.50) million motile sperm ($p < 0.001$) when compared to men with subclinical varicocele not undergoing surgical treatment [1]. While this is the best evidence to date, the variation in the studies makes it difficult to generalize this data (heterogeneity: $I^2 = 0\%$, $\tau\ 2 = 0$, $p = 0.82$) [1].

Pregnancy Rate

The primary goal of any study evaluating the efficacy of an intervention in fertility is ultimately the pregnancy rates. Pregnancy data is also the most difficult data to obtain. In a meta-analysis comparing treatment versus no treatment versus clomiphene citrate, in five studies the OR of pregnancy with treatment of varicocele was 1.29 (95%, CI 0.99–1.67 x^2 3.07, $I^2 = 0\%$) [25]. In another recent meta-analysis, correction of a subclinical varicocele was compared to correction of clinical varicocele and to observation. Data was collected from 13 studies and included 1357 men. In this analysis, the pregnancy rate of men with subclinical varicocele undergoing varicocelectomy compared with those who did not was 15% (95% CI 7.0–23.0%) vs 11% (95% CI 2.0–19.0%) ($p = 0.49$). When comparing men with clinical vs subclinical varicocele undergoing varicocelectomy, the annual pregnancy rate was 12% (95% CI 4.0–19.0%) for the clinical group vs 18% (95% CI 7.0–23%) for the subclinical group ($p = 0.18$) [1]. This analysis showed that there is likely some improvement in pregnancy rate when fixing a subclinical varicocele, though this is not as significant as the improvement with clinical varicoceles.

Conclusion

In patients with subclinical varicocele, the decision to operate is a difficult one: clinical benefit with correcting subclinical varicocele remains inferior to correcting a clinical varicocele [2], and thusly, the American Society for Reproductive Medicine recommends against the correction of subclinical varicocele [28]. However, the quality of the data is not robust and lacks large randomized controlled trials. Additionally, there is no standardization of the definition of a subclinical varicocele with some studies utilizing 2 mm and others 3 mm [3, 5, 25, 27, 29, 30]. Studies do not use a uniform approach to treatment [1, 25]. While there is data that repair of a subclinical varicocele while repairing a contralateral clinical varicocele may be helpful in some patient populations (Table 34.1), there is currently not enough evidence to recommend this to all patients. The potential benefits as well as added risks should be discussed with each patient individually.

Review Criteria

To determine the efficacy of unilateral versus bilateral ligation in patients with subclinical and clinical varicocele, we performed an exhaustive search utilizing multiple search engines such as OVID, MEDLINE, PubMed, and Google Scholar utilizing the search phrases "male infertility," "varicocele," "subclinical varicocele," "varicocelectomy," "varicocele embolization," "oligospermia," "semen analysis," and "pregnancy." All studies were evaluated regardless of date or language with priority for inclusion to be the most recent studies. Additionally, we reviewed the bibliographic contents of these articles to identify additional articles that our initial search did not include. We did not include abstracts or presentations.

Table 34.1 Effects of repair of subclinical varicocele

	Study	Surgical intervention	Subclinical varicocele definition	Treatment groups	Number of patients	Change in concentration (10^6)	Change in motility (%)	Total motile count (10 ^6)	Number of pregnancies (%)
Subclinical varicocelectomy vs no surgical treatment	Cantoro et al. 2015	Embolization	Not palpable during physical examination with one or more veins >3-mm diameters during Valsalva ultrasound	Subclinical vs control	218 vs 118	20.9 (+/−11.6) vs −0,9 (+/−8.6)	14.2(+/−14.2) vs 0.8 (+/−14.8)		100 vs 14 (46% vs 12%)
	Seo et al. 2010	Microsurgical inguinal ligation	Not palpable during physical examination with one or more veins >3-mm diameters during Valsalva ultrasound	Subclinical vs control (L carnitine)	25 vs 93	18.2 (+/−59.1 vs 1.2 (+/−57.4)	6.1 (+/−20.8) vs 0.4 (+/−30.8)		12 vs 19 (60% vs 35%)
	Unal et al. 2001	Inguinal ligation	Palpation negative diagnosed by color Doppler ultrasonography	Subclinical vs control (clomiphene citrate)	21 vs 21	11.9(+/−27.8) vs 7.5 (+/−11.7)	9.0 (+/−12.4) vs 15.1 (+/−21.2)		2 vs 1 (10% vs 5%)
	Yamamoto et al. 1996	Inguinal ligation	Palpation negative, thermal asymmetry greater than 0.3 C by scrotal thermography	Subclinical vs control	40 vs 73	5.9 (+/−10.9) vs −1.6 / (+/−4.2)	1.5 (+/−7.6) vs −0.1 (+/−2.6)		3 vs 4 (45% vs 40%)
Subclinical varicocelectomy vs clinical varicocelectomy	Alshehri et al. 2105	subinguinal ligation	Palpation negative, but vein diameter >2.5 mm by duplex ultrasound during Valsalva	Subclinical vs clinical	18 vs 39				3 vs 15 (17% vs 38%)
	Jarow et al. 1996	Embolization or ligation	Palpation negative, but vein diameter >2.7 mm by ultrasound during Valsalva. On venography contrast material refluxed down to the level of the inguinal canal	Subclinical vs clinical	37 vs 37			0.4 (+/−32.0) vs 25.0 (+/−23.7)	
	Dhabuwala et al. 1992	Inguinal ligation	Palpation negative but varicocele detected by Doppler examination		16 vs 38	14.4 (+/−16.5) vs 23.6 (+/−32.3)	−2.2 (+/− 3.3) vs 6.0 (+/−2.5)		8 vs 19 (47% vs 50%)

Multiple Choice Questions and Answers

1. Subclinical varicocele is most commonly discovered on:
   (a) Physical exam
   (b) Nuclear medicine scan
   (c) Venography
   (d) **Ultrasound**
2. Which of the following would *not* be a reason to fix a subclinical varicocele?
   (a) **Incidental subclinical varicocele in a fertile patient**
   (b) Subclinical varicocele in an infertile patient who has failed other treatments
   (c) Subclinical varicocele in highly motivated patient who also has a clinical varicocele on the other side
   (d) Subclinical varicocele in an infertile patient who is unable to have other fertility treatments
3. Which of the statements about subclinical varicocele and pregnancy rates is true?
   (a) Ligation of subclinical varicocele does not affect pregnancy.
   (b) Surgery of subclinical varicocele has an equivalent effect on pregnancy as does in a clinical varicocele.
   (c) **Subclinical varicocelectomy improves pregnancy rates, but less than with a clinical varicocele.**
   (d) Subclinical varicocelectomy decreases pregnancy rates.
4. Which statement is true about subclinical varicocele?
   (a) They always progress to a clinical varicocele.
   (b) They never progress to clinical varicocele.
   (c) **They can progress to clinical varicocele, but this is the minority of patients.**
   (d) Subclinical varicoceles are a different clinical entity than clinical varicoceles.
5. Patients with a subclinical varicocele should be counseled (check the best answer):
   (a) They are an important lesion that causes infertility in most patients.
   (b) **They may affect fertility, but this is not well defined at this time; ligation is only recommended in a few selected cases.**
   (c) They will lower testosterone if left untreated.
   (d) Ligation is never recommended.

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35 Is There Any Role for Intraoperative Ultrasound During Varicocele Repair?

João Paulo Cardoso
and Marcello Antonio Signorelli Cocuzza

Key Points

- Vascular anatomy of the subinguinal access represents a challenge and contributes to the technical difficulty of this approach.
- Preoperative parameters are not predictive of the number of testicular arteries or veins dissected and identified at the time of surgery.
- Optical magnification, microsurgery skills, and vascular Doppler should be offered to achieve maximal preservation of the arterial blood supply to the testes and correct identification of cord structures.
- Systematic use of intraoperative Doppler during subinguinal microsurgical varicocele repair allows a higher number of arterial branches preserved as well as a superior number of internal spermatic veins ligated.
- Recent studies show that the total number of veins ligated correlated with improvements in total sperm motility and sperm concentration.

Introduction

Infertility affects approximately 13–15% of reproductive-age couples. Male infertility underlies about 60% of the cases [1], directly or indirectly. Varicocele is detected in 35–50% of men with primary infertility and up to 81% of patients with secondary infertility [2–4].

Varicocele-induced testicular impairment has been widely studied, with no single theory yet to be considered the hallmark of this disease. Potential mediators in the pathogenesis are scrotal hyperthermia, backflow of toxic metabolites, testicular hypoperfusion, hypoxia, and hormonal disturbances. More recently, evidence suggests oxidative stress as an important feature in both physiological and deleterious processes of sperm capacitation, acrosome reaction, and signalling for fertilization [5].

Varicocelectomy has a main objective of disrupting the retrograde backflow from the abdomen to the pampiniform plexus and reducing the influence of mediators on the testicular function. Current data supports the statement that varico-

J. P. Cardoso · M. A. S. Cocuzza (✉)
Department of Urology, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, Sao Paulo, SP, Brazil
e-mail: mcocuzza@uol.com.br

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cele repair does indeed have a beneficial effect in reversing the harmful effects of varicocele upon testicular function in selected patients, by improving seminal parameters in the majority of controlled studies. Agarwal et al. [6] and Baazeem et al. [7] performed a meta-analysis which endorsed this beneficial effect. The reproductive outcome of the surgical procedure was also reviewed by Ficarra et al. [8], with higher pregnancy rates after varicocelectomy. Marmar et al. [9] found a 2.8-fold higher chance of spontaneous pregnancy after varicocelectomy.

In this chapter, we discuss the role of intraoperative ultrasound during the varicocelectomy, specially the microscopic subinguinal technique, where Doppler has its most common use. We provide the reader with current evidence related to Doppler technology for varicocelectomy.

Surgical Access and Techniques

Varicocele treatment has been described for centuries, with a variety of historical treatments that were mostly implemented for pain management. Surgical treatment of varicocele has changed dramatically over time since Palomo [10] first described his technique of high ligation proximal to internal inguinal ring in 1947. The procedure involves ligation of both spermatic artery and veins. Therefore, the cremasteric and deferential arteries remain the blood supply and was believed to provide sufficient inflow for the testicle.

Concepts and anatomy knowledge have changed over time, with evolution of diagnostic images and surgical techniques and introduction of microscopic assistance and artery-sparing principles. A diversity of open surgical techniques have been introduced, including retroperitoneal, inguinal, and subinguinal access. Possibilities of preservation of the internal spermatic arteries, vas deferens, and lymphatics vary between the techniques, since size, number, and location of vascular and nonvascular structures are variable depending on the level of the surgery.

Recently, open microsurgical inguinal or subinguinal varicocelectomy techniques have been shown to result in better pregnancy rates and fewer recurrences and postoperative complications than conventional varicocelectomy techniques in infertile men [11]. The subinguinal approach follows the same principles as the inguinal approach but is performed through an incision below the external inguinal ring, obviating the need to open the aponeurosis of the external oblique and resulting in less postoperative pain, as well as a shorter recovery period [12, 13].

Vascular Anatomy of Subinguinal Access

Three groups of veins form the pampiniform plexus. Dilated veins are usually found in the anterior group which is mainly composed of internal spermatic veins. Cremasteric or external spermatic veins form the posterior group, which may be dilated in some cases. The third group is located closely adherent to the vas deferens. The diameter of the internal spermatic veins is variable, from large and conspicuous in higher-grade varicoceles to small-diameter vessels in cases with subclinical or simple reflux [14].

Hopps et al. performed a prospective study in 48 patients, with a total of 84 microsurgical subinguinal varicocelectomies comparing them with 115 inguinal varicocelectomies that were previously performed. A detailed microanatomy of the spermatic cord at the subinguinal level was recorded using microscopic enhancement and a microruler for precise measurement. Researchers characterized a smaller number of large (greater than 5 mm) internal spermatic veins and a greater number of small (less than 2 mm) veins. Regarding external spermatic veins, those which did not accompany the cord vessels, the subinguinal approach showed a larger number of vessels greater than 2 mm. Furthermore, arteries were surrounded by a dense complex of adherent veins in 95% of the cases in subinguinal approach. This finding occurred in 30% of the cases with inguinal approach [13].

Therefore, vascular anatomy of the subinguinal access represents a challenge and contributes to the technical difficulty of this approach.

Branching of the testicular artery is usually seen in this level and therefore requires special attention intraoperatively.

Microscopic Approach to the Spermatic Cord

Microsurgical subinguinal varicocelectomy is the preferred approach endorsed by most experts. It was first introduced by Marmar et al. in 1985 [12], in an attempt to decrease complication and recurrence rates related to high ligation of internal spermatic veins via inguinal or retroperitoneal approach. Experience with microsurgical approach of the spermatic cord had been limited and considered complex and time-consuming. Marmar described a technique with a small incision just below the external inguinal ring, with microdissection of the spermatic cord, ligation of dilated veins, and treatment of small cross-collateral veins. The technique was further modified by Goldstein in 1992 [15]. Silber [16], in one of the first publications regarding microsurgical varicocelectomy, recommends caution with unintended ligation of the spermatic artery. Collateral circulation through cremasteric and deferential artery may not always be sufficient to maintain adequate testicular function. He also highlighted that most urologists performing varicocelectomy without magnification may not be able to identify the internal spermatic artery. An operating microscope allows for 6× to 25× magnification of the operating field. Preservation of the internal spermatic artery and lymphatic vessels and meticulous hemostasis are easily achieved, with the surgeon's visual acuity and precision enhanced [17]. Hence, this results in lower recurrence and hydrocele rates after the procedure, as well as avoidance of iatrogenic injuries [18, 19].

Preserving the testicular artery would also avoid damage to the seminiferous tubules that may occur even without testicular atrophy, as described in previous studies in both human and animal models. Researchers have demonstrated susceptibility of the testicular tissue to ischemia. Steinberger et al. [20], in a study on animal models dating back to 1879, demonstrated the destruction of most cells of the germinal epithelium following arterial ligation and interruption of blood flow to the testes. Spermatogonia, Leydig cells, and Sertoli cells all showed different degrees of susceptibility to induced ischemia.

Yaman et al. [21] conducted a retrospective study on 92 adolescents (age 11 to 19, mean 15.8) submitted to microsurgical subinguinal surgery for grade II and III varicocele. Doppler ultrasound probe was used in all procedures to identify and preserve the spermatic artery and lymphatics. There was no evidence of testicular loss or hypotrophy during the follow-up, with most of the patients demonstrating catch-up growth of the testicle after the procedure. Minevich et al. also performed a case series on adolescents, submitted to inguinal microsurgical varicocelectomy and Doppler assistance, describing prevention of hydrocele and varicocele recurrence [22].

Microscopic approach has also been used effectively for treatment of recurrent varicoceles. Grober et al. [23] performed a retrospective chart review of 54 patients who presented with persistent or recurrent varicocele, within a series of 1424 patients from a single surgeon. Treatment with microsurgical varicocelectomy in recurrent varicocele appeared to be safe. No varicocele persistence/recurrence, hematoma, or hydrocele was observed during follow-up, and outcomes were comparable to primary varicocele treatment in terms of semen parameters, serum testosterone, testicular volume, and pregnancy rates. Authors reported that the difference in incidence of recurrence after initial repair depends on the treatment modality which varied from 0.6 to 45%. The main cause for recurrence is the persistence of patent collateral veins missed during the original approach, although anatomical variation and ineffective venous ligation also contribute.

Therefore, microscopic approach has rendered varicocelectomy a more effective treatment. Precise identification of cord structures is important, especially when considering a variable number of spermatic arteries and uncertain contribution of collateral circulation. Optical magnification increases the chance of artery sparing and is safe for primary varicocele treatment

as well as for recurrence management, in both adults and adolescents.

Use of Intraoperative Doppler Ultrasound

Varicocelectomy requires meticulous dissection and identification of the vascular architecture of the spermatic cord, which demands knowledge of the testicular arterial anatomy. Studies have reported multiple spermatic arteries identified in approximately 40% of spermatic cords during microsurgical varicocelectomy at the subinguinal level [13, 24], increasing the technical difficulty of this approach. Although there is no agreement about the necessity to preserve all testicular arterial branches [25, 26], arterial ligation might be responsible for suboptimal spermatogenic recovery or failure to improve fertility in some cases.

Previous published data indicates that preoperative parameters are not predictive of the number of testicular arteries and veins identified at the time of surgery. Regarding anatomical parameters before surgery, in a prospective study of 65 varicocele units, Belani et al. [27] described larger internal spermatic veins in a grade 3 varicocele. Nonetheless, the study did not show statistically significant correlation in terms of number of veins dissected during the procedure and varicocele grade classification before surgery. In another study, Grober et al. sought to determine association between serum parameters and clinical varicocele classification with the number of arteries identified and preserved at the time of varicocelectomy. After 474 microsurgical spermatic cord dissections, analyzed data was not able to correlate preoperative parameters such as FSH, LH, testicular volume, and varicocele grade to the number of testicular arteries identified at the time of surgery [24]. The authors suggested that maximal preservation of the internal spermatic artery should be pursued despite the variable intraoperative arterial anatomy of the spermatic cord and negative findings of this study.

Lack of precise predictors of spermatic cord anatomy is compounded by the difficulties to accurately classify a tubular structure as an artery, a lymphatic vessel, or an adherent internal spermatic vein with certainty. As a result, advances in techniques of varicocelectomy, including optical magnification, microsurgery skills, and vascular Doppler, should be applied to achieve maximal preservation of arterial blood supply to the testes.

Recognition of the main spermatic artery can be confirmed by visualization of clear pulsatile movement and/or evidence of antegrade pulsatile blood flow with gentle lifting and partial occlusion of the vessel. However, identification of tiny secondary arteries is not at all times apparent. Consequently, it is possible that an inadvertent unrecognized ligation of a small internal spermatic artery occurs more frequently than reported [19]. Following are some of the reasons that could explain how spermatic artery injury may occur with optical magnification alone: First, the size of the arteries may be so small that the pulsation is difficult to identify. Second, aggressive manipulation of the vessels during dissection can lead to spasm, making it difficult to identify arterial pulsation. Third, the arteries tend to be in close proximity to or buried under complex branches of veins [19]. In all those situations, the use of vascular Doppler may help to preserve the arterial branches (Fig. 35.1).

The pulsation of internal spermatic artery can be seen with the use of microscope, once the vessel is isolated. Nonetheless, this pulsation can be

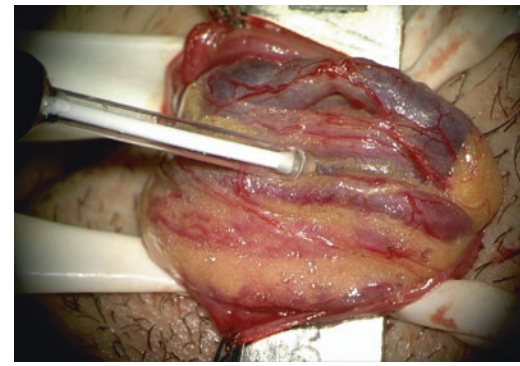

Fig. 35.1 Sterile Doppler probe in intraoperative use, microscopic enhancement, tip of the probe focused in a vessel within a branch of vascular structures

transmitted to the closely adherent veins and confuse the surgeon [14]. This is specially challenging in the subinguinal approach, where the internal spermatic artery is usually reduced in diameter. The surgeon must precisely ligate and cut the veins individually, without including adherent vessels. Vessel identification with Doppler should be performed in the beginning of the procedure, before excessive dissection of the spermatic cord. This is because arteries may spasm due to surgical manipulation, which decreases the chance of precise identification. A sterile disposable intraoperative probe attached to a 9.3 MHz VTI surgical Doppler (Vascular Technology Int., USA) (Fig. 35.2) is placed gently on the vessel, with the tip angled towards the patient's head. To increase sensitivity of vessel recognition, an acute angle between the probe and the vessel should be achieved, off the perpendicular angle of incidence. Angles between 30° and 60° are usually employed [28]. The higher the frequency of Doppler signal, more ultrasound beam is aligned to the direction of the flow. The sound frequency produced after probe placement upon a vessel is also proportional to the blood velocity inside the vessel.

Fig. 35.2 9.3 Mhz VTI surgical Doppler (Vascular Technology Int., USA) and a disposable probe flow detector

In 1981 and 1984, the first publications regarding the use of Doppler by Greenberg et al. [29] and Ramadan et al. [30] confirmed the valuable assistance this device offers in preservation of the spermatic artery. These publications are complemented by Wosnitzer and Roth's [14] study on subinguinal anatomy, as aforementioned in the anatomy section. Surgical Doppler allows identification and individualization of tiny vascular structures, by differentiating the blood flow through the small vascular structures. The surgeon can therefore decide more precisely which vessels should be ligated or preserved. A recent study Cocuzza et al. showed that concomitant use of intraoperative vascular Doppler during subinguinal varicocelectomy allows a higher number of arterial branches to be identified and therefore preserved [31]. Data concerning surgery with and without the use of Doppler vascular assistance show that a solitary artery is identified in 45.5% and 69.5% of cords, respectively, while two arteries are identified in 43.5% and 28.5%, respectively, and three or more arteries are identified in 11% and 2%, respectively. The authors also reported a higher number of internal spermatic veins were ligated when Doppler was used (Table 35.1). The use of intraoperative Doppler allows the surgeon to a higher confidence during dissection of a dense complex of adherent veins surrounding the artery in 95% of cases when the subinguinal approach is used [13]. Accidental artery ligation documented by a pulsatile twitching of the ligated vessel stump under magnification is less common when Doppler is applied [31].

The clinical implication of these findings was supported by recent studies showing that the total number of veins ligated was significantly positively correlated with improvements in total sperm motility and sperm concentration. Pasqualotto et al. conducted a prospective study of 62 patients and described higher improvement rates in sperm concentration when more than 10 veins were ligated [32]. Shindel et al. performed a retrospective analysis of left subinguinal varicocelectomies in 42 patients, describing positive correlation between number of veins ligated and increase in total motility [33].

Table 35.1 Intraoperative evaluation of internal spermatic veins ligated, number of lymphatic spared, and arteries preserved and injured in 377 spermatic cord dissections during microsurgical subinguinal varicocele repair with and without vascular Doppler

Variable	With Doppler (no. of spermatic cords = 225)	Without Doppler (no. of spermatic cords = 152)	*p* value
Number of veins ligated[a]	8.0 (3.1)	7.3 (2.8)	0.02
Number of arteries preserved[a]	1.6 (0.6)	1.3 (0.5)	<0.01
Number of arteries injured [b]	0	2 (1.1%)	0.06
Number of lymphatics spared[a]	2.2 (1.2)	2.0 (1.5)	0.21
Operative time unilateral repair (min) [a]	52.8 ± 17.8	53.0 ± 36.7	0.98
Operative time bilateral repair (min) [a]	101.0 ± 16.2	101.9 ± 16.3	0.37

Note: [a]Values are mean and SD. Compared using Student's unpaired *t*-test. [b]Data presented as number (percentage) of patients. Compared using Chi-square test. $P < 0.05$ was considered statistically significant
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More recently, Guo et al. [34] conducted a prospective randomized study comparing outcomes for microscopic subinguinal varicocelectomy with and without the use of Doppler ultrasound. One hundred seventy-two patients were strictly selected and randomized in two groups: simple microscopic varicocelectomy and intraoperative vascular Doppler ultrasound-assisted microscopic varicocelectomy. Each group was designated to a single surgeon. Procedures were performed by surgeons trained in microsurgery and male infertility, set in the same hospital and using the same technique. The difference between the groups was the systematic use of intraoperative Doppler to identify all vessels before ligation in the Doppler-assisted group. The number of internal spermatic veins ligated and the number of internal spermatic arteries preserved during surgery were significantly higher in the Doppler-assisted group. Operative time was shortened by the use of intraoperative Doppler, a finding the authors attribute to rapid and precise identification of vessels. No testicular atrophy was described. In terms of semen parameters, results showed improvements from the third month of follow-up and were durable at 6 and 12 months, with significantly higher sperm motility in the Doppler-assisted group. Pregnancy rates were not different between the two groups compared in the study, maybe due to sample size limitation.

Apart from reproductive outcomes, for adolescents, children, and patients with solitary testis, preserving the testicular artery is considered important [35]. As mentioned earlier, studies in adolescents with use of Doppler assistance have showed adequate outcomes, with catch-up testicular growth and lower rates of testicular atrophy.

In summary, as abridged in Table 35.2, recent evidence suggests that ligating a larger number of veins may achieve a better outcome in varicocelectomy by more effectively interrupting the refluxing venous drainage of the testis. Doppler assistance allows more certainty of arterial identification and vein ligation and safety of the procedure. Although the necessity to preserve testicular arteries is still controversial, attempts to preserve arteries during the procedure is recommended considering the arterial supply to the testes. This is especially important during varicocelectomy with subinguinal approach.

Conclusion

Vascular anatomy of the spermatic cord has always represented a challenge throughout history of the varicocele treatment. Nevertheless, the role of varicocelectomy in improving semen parameters and pregnancy rates has been described in many studies and meta-analyses, with subinguinal microsurgery being the preferred technique. Subinguinal vascular anatomy is especially challenging, considering the reduced size and increased branching of the internal spermatic artery at this level.

The use of intraoperative Doppler provides the surgeon higher confidence during dissection of a

Table 35.2 Summary of studies with intraoperative Doppler assistance

Author	Design	Number of patients	Technique	Study findings	Pregnancy outcomes
Greenberg, 1981	Editorial	N/A	N/A	Recommendation to use a method of artery identification with reference to intraoperative Doppler	N/A
Ramadan, 1984	Prospective	32	Open inguinal	An external Doppler was used to test arterial flow after vessel compression, before ligation	N/A
Minevich, 1998	Prospective	32	Inguinal microscopic	Reduced hydrocele and recurrence with use of microscopic and Doppler assistance in adolescents	N/A
Yaman, 2006	Retrospective	92	Subinguinal macroscopic	Majority of catch-up growth and no testicular atrophy in adolescents	N/A
Cocuzza, 2007	Prospective non-randomized	213	Subinguinal microscopic	Higher number of arteries identified and higher number of veins ligated with use of intraoperative Doppler	N/A
Shindel, 2007	Retrospective	42	Subinguinal microscopic	Positive correlation with number of veins ligated and increased motility	N/A
Gou, 2015	Prospective randomized	172	Subinguinal microscopic	Higher number of internal spermatic veins ligated and higher number of internal spermatic arteries preserved with better improvement in semen parameters of motility in the Doppler-assisted group	No significant difference

Note: N/A, not applicable

dense complex of adherent veins surrounding the artery, present in most of the cases. Although preservation of all testicular arterial branches is still a controversial topic, suboptimal spermatogenic recovery or failure to improve fertility in some cases might be due to reduced testicular blood supply.

Better identification of cord structures with the use of intraoperative Doppler ultrasound may allow a larger number of veins to be safely ligated during the procedure. A greater decrease in the reflux would in turn lead to diminished insult to spermatogenesis. This assumption is compatible with recent evidence pointing towards better outcomes in seminal motility and concentration. Nonetheless, studies have not yet demonstrated specific improvements in terms of pregnancy outcomes, a fact that may be attributed to limited sample sizes. Larger randomized studies may be able to positively correlate Doppler assistance to pregnancy outcomes in the future.

In conclusion, Doppler enables precision and safety to the surgical procedure. Recent evidence is consensual in recommending intraoperative ultrasound use as an important tool to improve outcomes of varicocele treatment.

Review Criteria

The current chapter is based on an electronic search using PubMed/MEDLINE database and references of the identified articles performed between March and May of 2018. The following keywords were used on the search engines: "varicocele," "Doppler,' "ultrasound," "varicocelectomy," "microscopic," and "microsurgical."

Multiple Choice Questions and Answers

1. The preoperative parameter that predicts the number of arteries identified during varicocelectomy is:
   (a) FSH
   (b) Varicocele grade
   (c) Testicular volume
   (d) **None of the above**

2. The use of intraoperative vascular Doppler has been associated with the following, *except*:
   (a) Higher sperm motility
   (b) Shorter operative time
   (c) **Higher rates of pregnancy**
   (d) Higher number of spermatic veins ligated
3. The use of intraoperative vascular Doppler has been associated with the following, EXCEPT:
   (a) Use in adolescents with lower rates of recurrence
   (b) **Higher number of lymphatic vessels preserved**
   (c) Higher number of spermatic veins ligated
   (d) Lower rates of accidental artery ligation
4. Microsurgical subinguinal varicocelectomy has become the preferred approach among experts for the following reasons, *except*:
   (a) Lower recurrence rates
   (b) Lower postoperative hydrocele rates
   (c) **Greater diameter of internal spermatic veins**
   (d) Less postoperative pain
5. The following are the reasons why spermatic artery injury may occur, *except*:
   (a) Arteries tend to be buried under complex branches of veins.
   (b) Arteries usually are small in diameter and pulsation may be difficult to identify.
   (c) Manipulation of the vessels during dissection can lead to spasm.
   (d) **Usually pulsation is not transmitted to close adherent structures.**

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36

Is There Any Role for Indocyanine Green Angiography in Testicular Artery Preservation During Microsurgical Subinguinal Varicocelectomy?

Chak-Lam Cho

Key Points

- Testicular artery injury is a major complication of microsurgical subinguinal varicocelectomy and is a potential cause of testicular atrophy. The incidence is unclear and probably underreported.
- Inspection of the cord for arterial pulsation under optical magnification with papaverine irrigation and utilization of micro-Doppler are the most widely adopted techniques for identification of testicular arteries currently.
- Indocyanine green angiography can be incorporated into the procedure of microsurgical subinguinal varicocelectomy with minimal preparation and training. The technique carries minimal risk.
- The high-contrast angiographic images of indocyanine green angiogram provides objective assessment of all testicular arterial flow. It is particularly useful for training and documentation.
- Intraoperative indocyanine green angiography is a useful adjunct to microsurgical subinguinal varicocelectomy. It facilitates earlier identification of testicular artery and potentially prevents testicular artery injury.

Introduction

Varicoceles are found in approximately 15% of the general adult male population [1]. The incidence rises to 35% in men with primary infertility and up to 80% in men with secondary infertility [2, 3]. Varicocelectomy is the most commonly performed surgery for the correction of male factor subfertility [4]. The goal of varicocelectomy is to ligate all venous drainage with the exception of the deferential veins. Complications arise when other important structures such as arteries, lymphatics, and nerves are accidentally injured. Various techniques and approaches have been described for varicocele repair [5]. Subinguinal approach allows exposure of external spermatic and gubernacular veins via a single incision, and the lack of fascial incision results in less pain postoperatively. These advantages make subinguinal varicocelectomy the preferred approach for many surgeons. On the other hand, the more difficult dissection with a greater

C.-L. Cho (✉)
S.H. Ho Urology Centre, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

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number of internal spermatic arteries and veins subinguinally poses challenges to the operating surgeons [6]. The application of operating microscope in varicocelectomy represents one of the major advancements by providing optical magnification which is essential for this meticulous procedure [7]. In view of the lower rate of recurrence and complications compared with open or laparoscopic techniques, microsurgical subinguinal varicocelectomy (MSV) has become the gold standard nowadays [8, 9].

Testicular Artery Injury

Testicular arterial injury is a known major complication of MSV and a potential cause of testicular atrophy, but the incidence is unclear [10]. Indeed, accidental arterial injury may go unnoticed and underreported particularly in non-microscopic varicocelectomy with inadequate optical magnification [11].

The preservation of testicular blood supply is a major concern during MSV. The notion is supported by the observation of testicular atrophy in animals after ligation of testicular arteries [12]. However, the debate between artery-preserving and artery-ligating approaches remains unsettled in humans. Testicular atrophy and azoospermia due to ischemia by testicular artery occlusion during varicocelectomy have been reported in humans with inadequate collateral arterial supply [13]. But a significant postoperative improvement in hormonal and semen parameters was still observed in the vast majority of patients with documented testicular arterial ligation from a large series [10]. Some authors advocated that the testicular artery may be sacrificed in order to achieve a complete venous ligation in patients with intact collaterals including cremasteric arteries, deferential arteries, or other distal collaterals [14]. Although the theoretical advantage of testicular artery preservation remains to be established, most urologists believe that maximal preservation of arterial supply to the testes is essential for the success of varicocelectomy. It was shown that the testicular artery supplies two thirds of the testicular blood supply [15]. Ligation of the testicular artery during MSV may affect spermatogenesis and may be a contributing factor in a decrease in postoperative natural pregnancy rates [10]. Therefore, every effort should be paid in preserving testicular blood supply especially when no branching of testicular artery was identified and the condition of distal collaterals was uncertain. It is of particular note that the deleterious effect of testicular artery ligation in a developing testis during adolescent varicocelectomy is unknown but potentially serious and irreversible.

Current Techniques of Testicular Artery Preservation and Limitations

Preservation of testicular blood supply in MSV consists of preservation of testicular, cremasteric, and deferential arteries. Currently, inspection of the cord for arterial pulsation under high magnification with papaverine irrigation and utilization of micro-Doppler are the most widely adopted techniques for arterial identification and preservation during MSV [16].

Most surgeons identify and protect the vas deferens together with its vessels and surrounding fascia during the initial part of the procedure. It is assumed that the deferential arterial supply is intact in patients without prior surgery which may damage collateral circulation distal to the external inguinal ring, for example, vasectomy, hernia surgery, and hydrocele surgery. Without further dissection of the vas deferens and deferential vessels, the status of the deferential artery is actually uncertain and not being assessed most of the time.

On the other hand, the preservation of testicular and cremasteric arteries may not be straightforward in many situations. Subinguinal approach is associated with a greater number of internal spermatic arteries and veins [6]. The smaller diameter of the arteries and higher likelihood of a dense network of adherent veins [6] suggest a more difficult dissection and challenging arterial preservation. An adherent bundle of vessels may dampen the arterial pulsation and the pulsation may not be evident until individual vessels are freely dissected from each other. The use of

micro-Doppler provides auditory signals reflecting the flow pattern of a vessel. The localization of characteristic arterial flow signal assists in differentiation between testicular artery and surrounding veins. However, the accuracy of micro-Doppler also depends on the direct contact between the probe and the artery and, therefore, successful lysis of adhesions among vessels. One anatomical study demonstrated that the testicular artery is adherent to the undersurface of a large vein in approximately half of the cases [17]. It means that the current application of operative microscope and micro-Doppler has their limitations and requires a certain level of experience in dissection of individual vessels. Successful preservation of testicular blood supply in MSV still largely relies on microdissection technique and is operator dependent.

Indocyanine Green Angiography in Microsurgical Subinguinal Varicocelectomy

Fluorescence imaging has its established clinical applications for many decades. Indocyanine green (ICG) angiography has been widely adopted in imaging of retinal blood vessels in ophthalmology with approval by the United States Food and Drug Administration since 1975 [18].

ICG is ideal for angiography by providing a good signal to noise ratio. The molecule binds efficiently to blood lipoproteins and produces a strong fluorescence of blood vessels against an almost black background. The short plasma half-life of 3 to 4 min allows repeated administration without compromising the image quality. ICG is a nonionizing and safe agent with severe adverse events reported in 0.05% of cases only, including anaphylactic reactions [19]. ICG imaging requires relatively simple optical instrumentation and recent developments in the technology mainly lie in the analysis of ICG fluorescence dynamics. ICG angiography can be easily incorporated into the intraoperative setting compared to other angiography modalities. It has been applied in neurosurgery, coronary surgery, reconstructive surgery, and trauma surgery where the status of blood circulation is of particular concern [20]. The use of ICG angiography has been expanded to minimally invasive urological procedures, for example, partial nephrectomy and prostatectomy [21]. The clear angiographic pictures provided by ICG angiography may facilitate the identification of testicular arterial supply during the procedure of MSV. The use of ICG angiography in MSV was reported and published recently [22, 23].

Preparation and Technique

Fluorescence angiography can be performed repeatedly during the procedure of MSV to differentiate between the arterial and venous nature of vessels under direct microscopic vision in the operating field. ICG angiography at the end of the operation has a role in confirmation of preserved arteries. Infrared fluorescence operative microscope and ICG are the additional equipment required for intraoperative ICG angiography. A pack of 25 mg of ICG was dissolved in 10 mL of water (Diagnogreen, Tokyo, Japan). Each angiography requires 5 mL (12.5 mg) of ICG solution which was prepared and administered by the anesthetist in a bolus via a peripheral line. The infrared mode of the microscope is activated and the fluorescence angiography is recorded and analyzed.

Interpretation of ICG Angiogram

Testicular arteries can be clearly identified by ICG angiography as demonstrated in Fig. 36.1. Small-caliber testicular arteries of less than 1 mm diameter can be visualized. In addition, cremasteric and deferential arteries are often shown up in most patients [23]. All arteries can be identified within 1 min upon ICG injection and testicular arteries are shown up with a mean time of 36 s [23]. Veins usually start lightening up 45 s after ICG injection with a much less intense fluorescence signal. Therefore, the interpretation of ICG angiography and identification

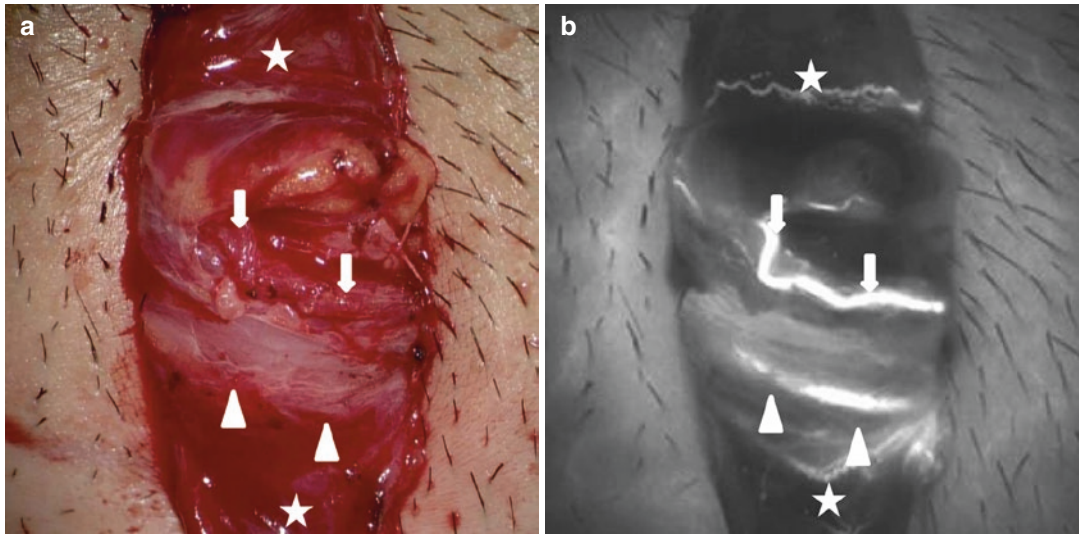

Fig. 36.1 Intraoperative images during microsurgical subinguinal varicocelectomy. (**a**) Microscopic view before injection of indocyanine green. (**b**) Indocyanine green angiography clearly demonstrated all the arterial supply to the testicle. The testicular artery was marked by arrows. Deferential artery and cremasteric arteries were denoted by arrowheads and stars, respectively. (Reprinted from Cho et al. [23]. With permission from Creative Commons Attribution License)

of testicular arteries is straightforward and requires minimal experience. The incorporation of ICG dynamics is made possible by a built-in computer program of certain operating microscopes (Fig. 36.2). The time to visualization and relative intensity of each vessel on ICG angiography can be assessed accurately and presented in different formats. Although the clinical significance of ICG dynamics is currently uncertain, the technology represents a potential tool in studying intraoperative vascular anatomy and physiology of varicocele.

Potential Advantages

There are several potential advantages in the use of ICG angiography as an adjunct in the procedure of MSV. ICG angiography has the capability in identifying even the smallest arteries. Testicular arteries with diameter 1 mm or less could be demonstrated clearly without ambiguity [23]. Cremasteric and deferential arteries could also be identified in most patients [23]. An intact deferential and cremasteric supply may predict less probability of testicular atrophy and impairment of spermatogenesis after testicular artery injury. Therefore, the confirmation of intact collateral supply achieved by ICG angiography may be preferable particularly in patients with prior groin or scrotal surgery when the status of collateral supply is doubtful. The technique of ICG angiography achieves complete assessment of testicular arterial supply which is not feasible with optical magnification and micro-Doppler. Ligation of dilated cremasteric veins can also be performed safely with clear identification of cremasteric arteries. Moreover, ICG angiography is unique in providing an objective real-time assessment of arterial flow in the cord compared to direct visualization of pulsation under high-power magnification and micro-Doppler. The technique is not operator-dependent. The intraoperative images can be recorded and are particularly useful for training and documentation purposes. It may facilitate transfer of technique to training surgeons and potentially shortens the learning curve.

The ease of adoption of ICG angiography represents another advantage. Minimal prior preparation of instruments is needed. The interpretation of high-contrast angiographic pictures

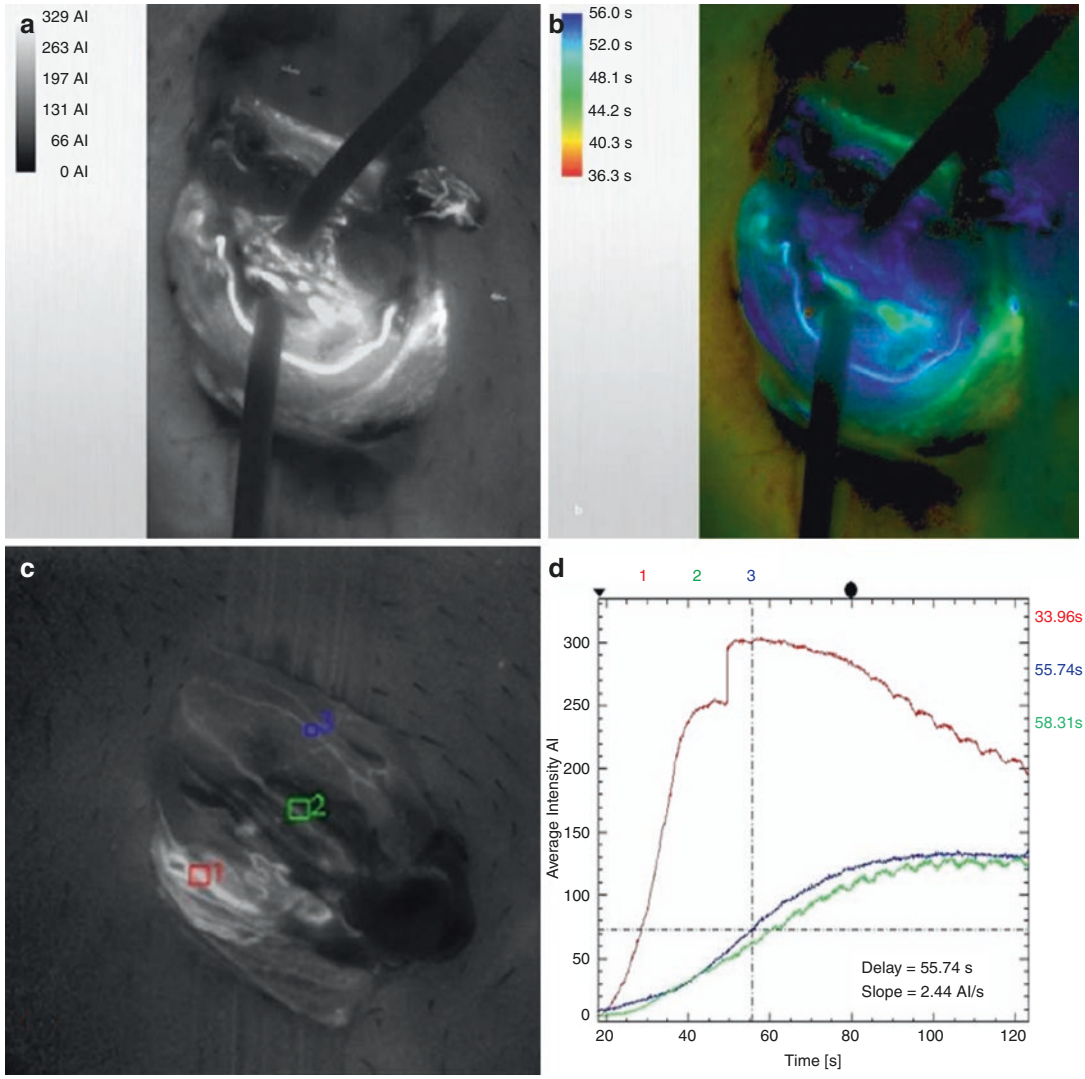

Fig. 36.2 Built-in fluorescence modules of the operating microscope (Zeiss OPMI Pentero 900, Oberkochen, Germany) provided analysis of the vascular dynamics. (**a**) Infrared 800 module demonstrates the relative intensity of indocyanine green signal. (**b**) Flow 800 module illustrates the sequences of the flow dynamics into a visual map. (**c**) Interpretation of specific area on the angiographic image can be marked. (**d**) Flow dynamic of each region can be illustrated in the form of curves. (Reprinted from Cho et al. [23]. With permission from Creative Commons Attribution License)

is straightforward even for novice surgeons. Each administration of ICG and capture of angiographic images spend no more than a few minutes and the technique does not significantly prolong the operating time. The short half-life and low risk of toxicity of ICG allows the angiography to be repeated every 15 to 20 min without compromising its ability for testicular artery identification. The technique of ICG angiography can be applied in adolescents with the same efficacy as in adults. The development of a computer software provides additional information on the dynamics of the arterial flow. The use of ICG dynamics could be a research tool to better understand the intraoperative microanatomy and physiology of varicocele. The advantages of ICG angiography in MSV are summarized in Table 36.1.

Table 36.1 Potential advantages of the utilization of indocyanine green angiography in microsurgical subinguinal varicocelectomy

Assessment of vasculature
Early localization of testicular arteries with minimal prior dissection and, thus, reduce the risk of accidental testicular artery injury
Identification of testicular arteries with diameter 1 mm or less
Assessment of all testicular arterial supply including testicular, cremasteric, and deferential arteries
ICG angiography
Provision of real-time imaging intraoperatively
Angiography in the form of pictures and videos are available
The technique is less operator-dependent
Interpretation of angiography requires minimal experience
The imaging provides objective documentation of testicular artery preservation
Excellent demonstration for training purposes
Potentially shortens the learning curve of novice surgeons and promotes patient safety during training
Setup of ICG angiography
Infrared fluorescence operative microscope and ICG are the only additional equipment required
Preparation and injection of ICG solution are simple
Injection of ICG is safe
ICG angiography does not prolong the procedure
The angiography can be repeated without compromising the image quality
Low running cost per procedure
Additional information
ICG dynamics may serve as a useful research tool in studying intraoperative vascular anatomy and physiology of varicocele
Confirmation of intact collateral testicular blood supply in patients having prior groin or scrotal surgery
Clinical applications
Particularly valuable in difficult situations when there is dense adhesion among intermingled arteries and veins
Adoption of the technique in both adults and adolescents
Localization of abdominal end of transected artery in case of accidental testicular artery injury

Clinical Applications

Despite the strong signal of testicular artery on angiography, a grossly dilated overlying vein may completely obscure the view. Certain degree of experience and microdissection technique is still necessary before the identification of testicular artery by ICG angiography in some patients. Therefore, the need for another adjunct in testicular artery identification during MSV is considered arguable by some surgeons.

Indeed, the use of ICG angiography may not be superior to direct inspection of arterial pulsation and micro-Doppler for simple cases. However, the technique enables earlier localization of testicular arteries and minimizes the extent of dissection. A tiny window exposing testicular arteries in the whole microscopic field is sufficient for the surgeon in picking up the strong arterial signal on ICG angiography. The adjunct is especially valuable for difficult scenarios in patients with dense adhesion among intermingled arteries and veins (Fig. 36.3). The adhesion renders the identification of testicular artery impossible by damping the pulsation. Exact localization of the weak pulsation by direct inspection may be difficult before the vessels are freely separated. When the technique of micro-Doppler mandates a larger window to access the artery by allowing direct contact with the probe, the extent of exposure required for accurate assessment by ICG angiography is much less. The lesser magnitude of mobilization before identification of testicular arteries by ICG angiography will reduce the chance of testicular artery injury during dissection and potentially leads to better preservation of arterial supply to the testicle. In addition, ICG angiography may have a role in testicular artery repair by localizing the abdominal end of the transected artery in case of accidental testicular artery injury.

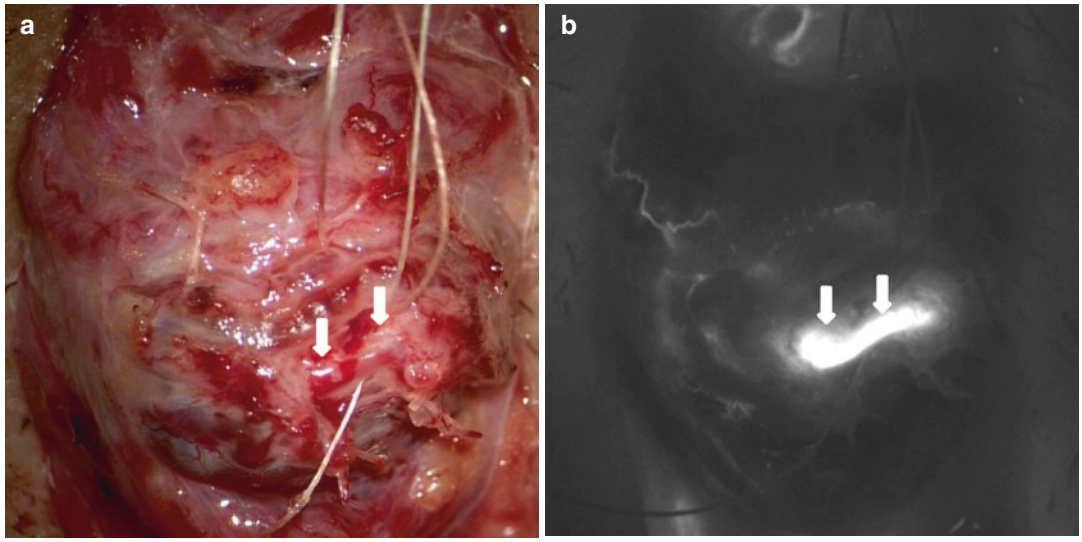

Fig. 36.3 Intraoperative indocyanine green angiography may facilitate early identification of testicular artery. (**a**) Microscopic view showing dense adhesions among intermingled artery and dilated veins which render the identification of arterial pulsation extremely difficult. (**b**) Indocyanine green angiography showed a single testicular artery among the densely adhered vessels. (Reprinted from Cho et al. [23]. With permission from Creative Commons Attribution License)

Cost-Effectiveness

The use of ICG angiography may prove to be more cost-effective than the use of other adjunct such as micro-Doppler. Although the setup of an infrared fluorescence operative microscope is more costly compared to a micro-Doppler machine (USD $283,000 versus $11,600), the microscope can be shared among different specialties in the setting of a multidisciplinary hospital. The running cost of ICG angiography is much lower than micro-Doppler for each procedure. A pack of 25 mg Diagnogreen costs around USD $43 in our locality and usually one to two packs were consumed for each procedure while a disposable micro-Doppler probe costs USD $386.

Novel Platforms

ICG-enhanced fluorescence imaging is not only limited to the platform of operating microscope. The technique has been incorporated into various laparoscopic procedures [24]. Visualization of spermatic cord structures is provided by a high-definition stereoscopic camera connected to a zero-degree scope. The 10 mm scope is equipped with a specific lens and light source emitting both visible and near-infrared light (Karl Storz, Tuttlingen, Germany). The system of camera and scope is held by a holding arm which provides steady image throughout the procedure. Magnification up to 8 times can be achieved with the current setting. The surgeon and assistant operate by looking into high-definition two-dimensional (2-D) television screens as in conventional laparoscopic surgery. The incorporation of a second video telescopic operative microscope (VITOM, Karl Storz, Tuttlingen, Germany) is feasible and can offer 16 to 18 times optical magnification comparable to operative microscope with high-definition 3-D images. It preserves the advantages of 3-D images offered by an operative microscope with addition of an ICG angiography feature by switching between the two telescopes. The system potentially provides a more ergonomic working environment compared to an operating microscope. Posture of operating surgeons is not limited by the eyepieces of the microscope as in standard MSV. After injection of ICG, the system projected high-resolution real-time images of angiography (Fig. 36.4).

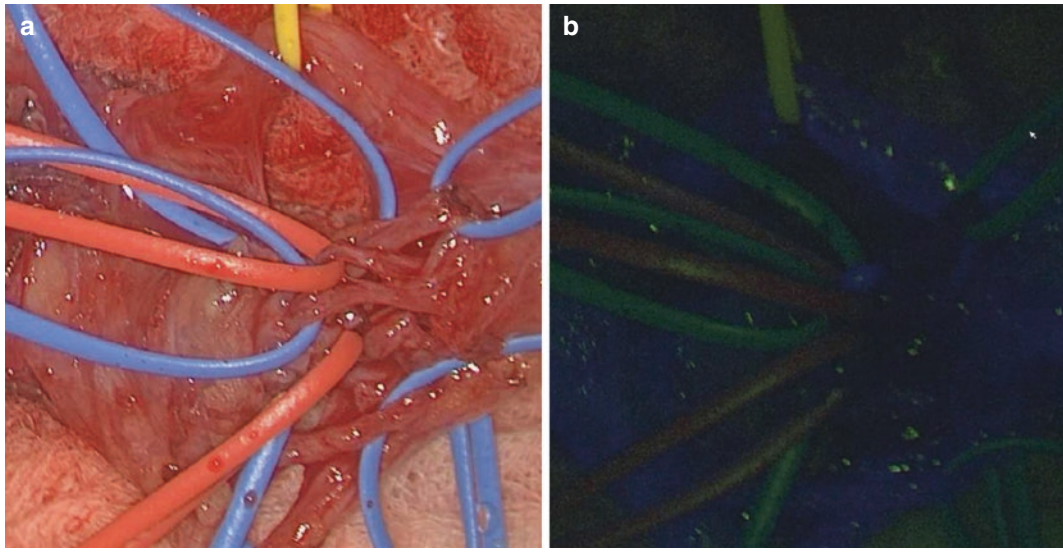

Fig. 36.4 Intraoperative images captured by high-definition stereoscopic camera connected to a zero-degree scope with near-infrared fluorescence emission (Karl Storz, Tuttlingen, Germany). (**a**) Microscopic view before injection of indocyanine green. (**b**) Arteries appeared blue upon injection of ICG

The robotic system with the da Vinci platform (Intuitive Surgical Inc., Sunnyvale, CA, USA) has been introduced in microsurgery including MSV [25]. The use of telescope-based high-definition video system (VITOM, Karl Storz, Tuttlingen, Germany) has been introduced. The use of VITOM can be integrated into the robotic TilePro system (Intuitive Surgical Inc., Sunnyvale, CA, USA) and supplements the 12- to 15-time digital magnification on the robotic system. The VITOM can be positioned on an additional fifth robotic (nitrogen powered, Karl Storz, Tuttlingen, Germany) arm. The setting currently provides 3-D high-definition images with high magnification. The incorporation of ICG fluorescence imaging into the system is still awaited.

Future developments in surgical video imaging may offer innovations to modify the procedure of varicocelectomy. All-in-one three-dimensional (3-D) imaging systems with high magnification and ICG fluorescence imaging will definitely improve surgeon performance and patient outcome. The platform of MSV may extend from the use of an operating microscope to other novel settings including open/laparoscopy and robotics. The new working platforms may provide higher image quality to surgeons in addition to better ergonomics.

Conclusion

The use of intraoperative ICG angiography is safe and provides an objective assessment of testicular arterial supply. The technique facilitates testicular artery preservation and may decrease the incidence of testicular artery injury during MSV. ICG angiography is potentially superior to and provides additional information compared to the current techniques of testicular artery identification with direct visualization of pulsation under high-power magnification and micro-Doppler ultrasonography. The adjunct may prove to be particularly useful in difficult scenarios with dense adhesion among vessels.

Review Criteria

An extensive search investigating the relationship between varicocele repair and testicular artery injury was performed using

search engines including ScienceDirect, OVID, PubMed and MEDLINE. The study identification was based on the following keywords: "varicocele," "varicocelectomy," and "testicular artery." The start and end dates for the searches were January 2000 to January 2018, respectively. Only articles published in English were considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included.

Multiple Choice Questions and Answers

1. Testicular artery injury:
   (a) Is a minor complication of varicocelectomy
   (b) Results in hydrocele
   (c) **Is reduced with the use of operative microscope**
   (d) Is less likely to occur with subinguinal approach
2. Inspection of the cord for arterial pulsation under high-power magnification with papaverine and utilization of micro-Doppler:
   (a) Identify all the potential testicular arterial supply
   (b) **Are operator-dependent**
   (c) Can replace microdissection technique
   (d) Provides visual images for identification of testicular arteries
3. Indocyanine green:
   (a) Binds efficiently to hemoglobin
   (b) Has a long half-life in plasma
   (c) Causes mild allergic response in 1% of patients
   (d) **Has a good signal to noise ratio for angiography**
4. The use of indocyanine green angiography during microsurgical subinguinal varicocelectomy:
   (a) **Requires the use of infrared fluorescence operative microscope**
   (b) Requires continuous infusion of indocyanine green via a peripheral vascular access
   (c) Prolongs the operation
   (d) Needs extensive experience in interpretation of angiographic images
5. Indocyanine green angiography is a useful adjunct to microsurgical subinguinal varicocelectomy due to the capability in:
   (a) Identification of large lymphatics
   (b) Identification of internal spermatic veins
   (c) Identification of testicular arteries larger than 1 mm in diameter
   (d) **Identification of testicular arteries early in the procedure**

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37 Should a Varicocele Be Repaired Before Assisted Reproductive Technology Treatment?

Eric Chung

Key Points

- Varicoceles are a major cause of impaired spermatogenesis and remain the most common correctable cause of male infertility. There is a potential risk for progressive fertility decline due to oxidative stress and sperm DNA fragmentation in untreated varicocele.
- Major reproductive organizations advocate an active role for varicocele management in infertile couples when the male partner has at least one abnormal semen parameter.
- Microscopic varicocelectomy is accepted as the standard of care with higher clinical outcomes and least complication rate.
- Varicocele surgery is likely more cost-effective than IVF/ICSI and has been shown to improve semen parameters, pregnancy rates, and live birth rates. However, in men with non-obstructive azoospermia, proceeding immediately to microTESE might be a more financially viable option.
- In couples who need ART, varicocele repair may offer improvement in semen parameters and sperm health that can increase the likelihood of successful ICSI/IVF fertilization and may decrease the need for further ART to achieve a successful pregnancy.

Introduction

Infertility affects up to 15% of couples and approximately half of cases are related to male infertility. Varicoceles are a major cause of impaired spermatogenesis and remain the most common correctable cause of male infertility [1]. Varicoceles are dilated spermatic cord veins of the pampiniform plexus and can be diagnosed by clinical palpation on physical examination, while the diagnosis of subclinical varicoceles requires color Duplex ultrasonography. It is thought that varicoceles commonly develop during puberty because of the differential growth of the testis and spermatic cord structures. Several proposed theories for varicoceles include the presence of incompetent venous valves with ensuing retrograde blood flow, a higher venous pressure in the left testicular vein due to the insertion of the vein at a 90° angle into the left renal vein, and/or left renal vein compression between the superior

E. Chung (✉)
AndroUrology Centre, Spring Hill, QLD, Australia

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mesenteric artery and aorta (the "nutcracker effect") that limits the venous outflow [2].

Regardless of their origin, varicoceles have been demonstrated to affect all components of the testis structures, namely, Leydig, Sertoli, and germ cells, thereby affecting spermatogenesis and hormone production and function [3]. While the exact pathophysiology on testicular dysfunction is yet to be fully established, several mechanisms have been proposed to explain its adverse impact on spermatogenesis such as testicular blood stasis, testicular venous hypertension, elevated testicular temperature, increase in spermatic vein catecholamine levels, testicular under-perfusion, and elevated oxidative stress [4]. The persistence of metabolic by-products that would typically have been quickly removed from the testes due to reflux from renal and adrenal metabolites has also been thought to have a negative effect on testicular function [5]. Furthermore, increased oxidative stress and thermal damage to the DNA and proteins in the nucleus of spermatic and tubule cells and/or Leydig cells with subsequent germ cell apoptosis may alter semen parameters and result in severe oligozoospermia or even azoospermia in the long term [6].

Varicoceles can be diagnosed in 40% of men with primary infertility and 80% of men with secondary infertility, although 12% of men with normal semen parameters may also have varicoceles [1, 7]. While it is thought that up to 5% of men with non-obstructive azoospermia (NOA) have varicoceles, its causative role in azoospermia is not fully elucidated. The impact of varicocelectomy on improving sperm production and fertility has been established since the early twentieth century [8]. Tulloch was one of the first surgeons to [9] demonstrate that high ligation of the spermatic vessels can improve fertility, and since that time, numerous studies have shown that varicocele repair is an effective treatment for male infertility. Both the American Society for Reproductive Medicine [10] and the European Association of Urology Guidelines on Male Infertility [11] advocate an active role for varicocele management in infertile couples when the male partner has at least one abnormal semen parameter.

The following chapter evaluates the clinical evidence pertaining to varicocele repair in improvement of semen parameters (including sperm retrieval rate), pregnancy rate in natural conception vs. assisted reproduction technology (ART), and cost analysis of varicocele surgery in the setting of couples who are undergoing in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatment.

Varicocele Surgery: Clinical Outcomes in Spermatogenesis, Pregnancy Rate, and Role in Assisted Reproductive Technology

Over the last 2 decades, there have been several controversies regarding the role of varicocelectomy in men with subfertility (or infertility). One of the earliest systematic reviews on the role of varicocele repair for male infertility published in 2003 [12] showed that varicocele repair was not an effective treatment for male infertility. However, this review was marred by many issues including heterogeneous data set with the inclusion of patients with non-palpable varicoceles and/or normal semen parameters which would have masked the actual benefits of treatment for clinically apparent varicoceles in men with impaired semen parameters. More recently, several meta-analyses that excluded men with subclinical varicoceles or normal semen parameters have confirmed the clinical efficacy of varicocele repair [13–15]. A more recent randomized, controlled trial published by Abdel-Meguid in 2011 [16] reported that varicocelectomy was associated with natural pregnancy rate of 13.9% in the observed arm and 32.9% after varicocele repair, with an odds ratio of 3.04 (95% confidence interval [CI], 1.33–6.95) and that the number needed to treat was 5.27 (95% CI, 1.55–8.99) to achieve natural pregnancy after varicocele repair.

On the other hand, Pasqualotto [17] did not demonstrate a significant difference in pregnancy rate after ICSI between couples who had a clinical varicocele and couples who had a varicocele repair (pregnancy rate was 31.1% vs. 30.9%,

P = 0.98). One of the major criticisms of this study was how the study defined pregnancy rate (i.e., the visualization of a gestational sac by ultrasound at 7 weeks) which was a much less clinically relevant outcome than live birth rate. The principle that pregnancy rate cannot be directly extrapolated to live birth rate was also underscored by another study in which Mansour [18] found that pregnancy rate was 44.1% in the group who underwent surgical repair and 19.1% in the expectant management group (P = 0.001), and of the pregnancies, there was a significant difference in miscarriage rate that further favored the varicocele repair group (13.3% vs. 69.2%, P = 0.001) beyond the initial outcome of clinical pregnancy that would no doubt lend itself to improved live birth rate in the treated group.

An important factor to consider when comparing the clinical outcomes between varicocelectomy and expectant management is the selection of subjects, especially since the bulk of the literature is retrospective and nonrandomized in study design. The potential bias from nonrandomized and inferior studies does not allow for definite conclusions regarding the efficacy of varicocele repair versus expectant management in pregnancy or live birth rate. Zini [19] reported that in men for whom pregnancy outcome was documented, there was no significant difference in natural or ART-assisted pregnancy rate between the two groups. However, the two groups were very different with the varicocele repair group having significantly worse semen parameters, decreased testicular volume, and significantly more men with primary infertility than the expectant management group. It can be argued that because the group that chose surgical repair had notably decreased baseline function, the varicocele repair was able to overcome this baseline deficit and equalize the couple's chance of pregnancy to those who have less severe dysfunction.

The Cochrane review published in 2012 reported that varicocelectomy has a combined fixed-effect odds ratio for the outcome of pregnancy at 1.47 (95% CI, 1.05 to 2.05, very-low-quality evidence), favoring surgical intervention [20]. The number needed to treat for an additional beneficial outcome was estimated at 17, suggesting the modest advantage of varicocele treatment over expectant management for pregnancy rate in subfertile couples in whom varicocele in the man was the only abnormal finding. Further subgroup analysis appeared to favor varicocele treatment, with a combined odds ratio of 2.39 (95% CI 1.56 to 3.66) with the number needed to treat for an additional beneficial outcome as 7. However, the evidence was far from conclusive as the quality of the available evidence was poor and the authors concluded that more research is needed with regards to live birth or pregnancy rate as the primary outcome.

Although the clinical effectiveness of varicocele repair appears substantial, the question of its cost-effectiveness should be addressed in the current era of ART since IVF/ICSI has been shown to be an effective treatment of male infertility and provides many infertile couples with a promising avenue to biological parenthood, by bypassing natural barriers to fertilization and in men with varicocele. The cost of the various ART procedures is an important financial consideration for couples (and society) given the economic burden as private and public insurance coverage was not universal and there is wide variability in the ART cost and its cost-effectiveness when comparing various ART procedures [21]. Chambers showed that the direct cost of one ART cycle in the USA was substantial and the out-of-pocket expense to cover these direct costs varied considerably across various states and insurance providers in the USA. Importantly, these cost estimates did not account for other indirect costs associated with ART such as loss of work productivity, incomes, unexpected cost of managing ART complications (e.g., ovarian hyperstimulation syndrome), or the financial burden of multiple gestation births [22]. The rising cost of ART is not unique to the USA alone, and many countries face substantial cost associated with ART even with generous government funding [23, 24]. Furthermore, there are many issues relating to ICSI pregnancies such as a 1.5–4-fold increases in chromosomal abnormalities, imprinting disorders, autism, intellectual disabilities, and birth defects when compared with pregnancies resulting from conventional IVF [25, 26]. It is common

for IVF/ICSI to be associated with an increase in twin pregnancy; the international twin birth rate is 20% after ART compared to 2% after natural conception [27]. Multiple gestations invariably pose a greater risk to the mother and have an increased rate of premature delivery and perinatal mortality compared to their singleton counterparts, thereby compounding the cost of prenatal care as well as neonatal care [28]. The increased economic cost of twin and higher-order births continues lifelong as these children face significantly increased long-term morbidities that are related to preterm delivery or during labor. The direct and indirect costs of ART have been a major driving force behind its lack of widespread adoption and utilization across the world.

In a cost-effective model of varicocele and IVF/ICSI, Schlegel [29] demonstrated that the cost per delivery per varicocele repair was remarkably less than the cost per delivery with ICSI (the average cost per live birth was $89,091 for IVF/ICSI and $26,268 for varicocele repair) and advocated that varicoceles associated with suboptimal semen parameters and infertility should be treated because this intervention provided better cost-effectiveness ratio per live birth when compared to ICSI. In a more elaborate cost-analysis study, Penson [30] compared the cost-effectiveness of four possible treatment strategies for infertility related to varicocele, namely, (1) observation, (2) varicocele repair followed by up to three IVF cycles if the couple did not conceive in the year after varicocelectomy, (3) three cycles of ovarian stimulation and intrauterine insemination (IUI) followed by three cycles of IVF if the IUI failed, and (4) up to three cycles of immediate IVF, and found that clinical observation was associated with 14% live births only and that proceeding directly to IVF was the least cost-effective management of infertility when the outcome measured is cost per live delivery. In addition, immediate IVF was only 61% effective, making this strategy more expensive coupled with a less effective outcome when compared to either immediate varicocele repair or IUI. Importantly, the probability of live delivery for varicocele before IVF was on par with IUI before IVF at 72% and 73%, respectively. The average cost per live delivery of the varicocele group was $32,171, while the average cost per live delivery of the IUI group was slightly higher at $36,322. Since the authors did not include indirect costs associated with ART in their analysis, it is likely that the cost benefit of varicocele repair is underestimated because the indirect costs of IUI/IVF are far greater than the indirect costs related to varicocele repair. In another decision analysis model for infertile couples with varicocele, Meng [31] showed that varicocele repair was more cost-effective than ICSI when men had a preoperative total motile count of <10 million sperm. When men had a total motile count of >10 million sperm, and thus qualified for IUI, varicocele repair was only more cost-effective than IUI when the postoperative pregnancy rate was >45%.

However, the role of varicocele repair in men with NOA is not clear. The return of viable sperm to the ejaculates of NOA men following varicocele repair is important as it alters the ART needs. It allows NOA men to attempt biological parenthood and potentially avoids the need for sperm retrieval. A recent meta-analysis [32] concluded that men with NOA had a 6% natural pregnancy rate after varicocele repair and that 39% of previously azoospermic men had a return of motile sperm to their ejaculate after varicocele repair, thus precluding the need for sperm retrieval procedures such as micro-TESE. The finding of sperm in the ejaculate after varicocelectomy has prompted further debate if varicocele repair should be advocated in men with varicoceles and NOA as initial therapy, or whether an immediate attempt at sperm retrieval via micro-TESE is a better alternative. Lee [33] has shown on a cost analysis study that proceeding directly to microTESE was more cost-effective and that varicocele repair should be deferred if the couple was to proceed to IVF/ICSI. The study showed that varicoceles repaired in azoospermic men would have to result in a 40% spontaneous pregnancy rate to be favored over proceeding directly to micro-TESE. Considering the documented 6% spontaneous pregnancy rate for azoospermic men who undergo varicocele repair, immediate microTESE proves to be a more financially viable option. On the other hand, Esteves [34] reported that approximately 44% of the treated patients will have enough sperm in postoperative ejaculate to allow ICSI to be

performed without the need for sperm retrieval. It has been suggested that fresh ejaculated sperm may yield superior ICSI success rates compared to sperm harvested by sperm retrieval. In addition, it was easier for the laboratory to handle such specimens [35]. Hence, whenever possible, it is preferable to use viable sperm from a fresh ejaculate than testicular sperm extraction in preparation for IVF/ICSI. Moreover, when sperm retrieval is required, the success of sperm retrieval will be improved following varicocele repair. The question of whether varicocele repair is more cost-effective is likely dependent upon the degree of abnormality in the semen parameters. There is clearly an increase in natural pregnancy rate after varicocele repair in men who have suboptimal semen parameter [36]. While the cost of including the varicocele procedure with IVF is obviously greater than with a single cycle of IVF alone, the increase in ART success will likely prevent the need for multiple rounds of IVF, supporting the role for concurrent varicocele repair with ART as a financially responsible option.

Since varicoceles can occur coincidentally in men with NOA, it is essential to rule out other factors that can contribute to spermatogenic disruption, such as Y chromosome or AZF microdeletions, before considering varicocele repair in these patients. It is important to note that there is a gradual decline in spermatogenesis and return to azoospermia in previously NOA men with varicocele beyond 1 year after varicocelectomy, making the long-term benefit of varicocele repair in this population unclear [37]. Given that relatively few men experience return of spermatogenesis following varicocelectomy and a significant proportion of these lose their spermatogenic capability, sperm cryopreservation is often recommended following initial improvement after varicocelectomy in these men.

An often overlooked consideration in the discussion of varicocele repair is the potential for progressive fertility decline if varicocele goes unrepaired. Reichart [38] showed quantitatively that there was a significant increase in normal acrosome structure, chromatin condensation, and sperm head appearance in men with treated varicoceles, but semen parameters were unchanged between groups, implying that ultramorphology may be a more sensitive means to assess sperm pathology in men with varicocele. On a molecular level, reactive oxygen species are elevated both in the semen and systemically in men with varicocele, and surgical repair results in decreased ROS, higher antioxidant levels, and lower DNA fragmentation. Furthermore, there is increasing evidence to suggest that sperm DNA fragmentation is associated with a higher risk of miscarriage in ART [39–41]. Meta-analysis on the effect of sperm DNA fragmentation on miscarriage rates found the risk of miscarriage was increased by 2.16-fold when semen specimens with an abnormally high proportion of DNA damage were used for ICSI (95% CI, 1.54–3.03, $P < 0.00001$) [42].

Conclusions

The definition of clinical success following varicocele repair has been a moving target that warrants serious clinical and financial consideration. Even though varicocelectomy may be successful in removing dilated veins of the spermatic cord, clearly the outcome of interest is related to enhancing underlying fertility potential. While varicocele surgery is advocated by all major reproductive organizations to improve semen parameters, sperm DNA damage, and changes to the seminal milieu in subfertile (or infertile) men, this may not be a clinically significant outcome that is relevant to infertile couples if the improvements do not translate to improved pregnancy rate, live birth rates, or enhanced ART outcomes (see Table 37.1). Current literature suggests that varicocele repair is a cost-effective treatment modality that can result in improvement in semen parameters, pregnancy rates, and live birth rates for most infertile males with clinical varicocele when there is no female infertility risk factor. Further studies are required to evaluate the impact of varicocele surgery in testicular endocrine function and sperm ultrastructure. In couples who need ART, varicocele surgery may offer improvement in semen parameters and sperm health that can increase the likelihood of successful IVF/ICSI and decrease the need for further ART cycles to achieve a successful pregnancy.

Table 37.1 Pros and cons comparing varicocelectomy vs. assisted reproductive technology

	Varicocelectomy	Assisted reproductive technology (ART)
Pros	1. Treats other varicocele-related problems (pain, cosmesis, etc.) 2. Improves semen parameters (count, motility, morphology, DNA fragmentation, etc.) and sperm retrieval rate 3. Increases natural pregnancy rate and success rate with assisted reproductive technology (downgrade ART or may avoid need for IVF/ICSI) 4. More cost-effective 5. Improves testosterone production	1. Varicocelectomy may not improve success rate (given other factors such as female issues) 2. Avoid delay starting a family 3. MicroTESE is more suitable (and cost-effective) in men with non-obstructive azoospermia
Cons	1. Delays time to IVF/ICSI 2. Surgical risks associated with varicocelectomy (pain, infection, recurrence, etc.) 3. Microvaricocelectomy is a complex microsurgery and may not be widely available	1. Does not address varicocele 2. Likely costlier

Review Criteria

An extensive search of studies examining the relationship between varicocele and assisted reproductive technology was performed using Google Scholar, PubMed, and MEDLINE. All English language original and review articles on varicocelectomy and male fertility over the last 20 years were conducted, and published guidelines from international organizations were included too. The overall strategy for study identification and data extraction was based on the following keywords: "varicocele," "azoospermia," "oligospermia," "microTESE," "cost-effectiveness," "successful pregnancy," and "assisted reproductive technology."

Multiple Choice Questions and Answers

1. Which of the following is not a reason for varicocele development?
   (a) Differential growth of the testis and spermatic cord structures during puberty
   (b) Presence of incompetent venous valves with ensuing retrograde blood flow
   (c) Higher venous pressure in the left testicular vein due to the insertion of the vein at a 90° angle into the left renal vein
   (d) **Compression of testicular vein from hydrocele**
   (e) Compression of left renal vein between the superior mesenteric artery and aorta (the "nutcracker effect")
2. Varicocele can affect spermatogenesis because:
   (a) **It increases testicular blood flow.**
   (b) It causes testicular venous hypertension.
   (c) It decreases intra-testicular temperature.
   (d) It decreases catecholamine levels in spermatic vein.
   (e) It minimizes oxidative stress within the testis.
3. The Cochrane review published in 2012 reported that:
   (a) Varicocelectomy lowers the odds ratio for the outcome of pregnancy.
   (b) **The number needed to treat for an additional beneficial outcome for pregnancy rate in subfertile couples in whom varicocele in the man was the only abnormal finding was estimated at 17.**
   (c) Varicocelectomy increases the risk for ART utilization.
   (d) The improvement in semen parameters is transient.
   (e) Varicocelectomy does not change fertility or pregnancy rate in subfertile couples in whom varicocele in the man was the only abnormal finding.
4. The clinical effectiveness of varicocele repair in the setting of ART:
   (a) Varicocelectomy is more expensive than IVF/ICSI.

(b) Varicocelectomy does not change the outcome of IVF/ICSI.
(c) Varicocelectomy delays the couple for successful pregnancy.
(d) **Varicocelectomy provides higher cost-effectiveness ratio per live birth when compared to ICSI/IVF.**
(e) Varicocelectomy is inferior to ICSI/IVF in pregnancy rate.

5. With regard to the role of microTESE in men with varicocele
(a) MicroTESE is less cost-effective than varicocele repair.
(b) There is higher spontaneous pregnancy rate in men who chose microTESE over varicocele repair.
(c) **Spontaneous pregnancy rate for azoospermic men who undergo varicocele repair is more than 10%.**
(d) Sperm harvested from varicocele repair is not superior to those obtained from microTESE.
(e) Varicocele repair does not improve the sperm retrieval rate.

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38 Should a Varicocele Be Repaired in Non-infertile Patients with Hypogonadism?

Taha Abo-Almagd Abdel-Meguid Hamoda

Key Points

- Varicocele may impair both spermatogenesis and steroidogenesis. Evidence indicates that varicocele may insult Leydig cells and links varicocele to impaired testosterone biosynthesis and hypogonadism.
- Testicular hyperthermia was proposed as a pathophysiological mechanism, though a multifactorial mechanism is more likely.
- Evidence indicates that varicocelectomy improves serum testosterone and could reverse hypogonadism among infertile men. These improvements inversely correlate to baseline serum testosterone levels, with baseline hypogonadal patients demonstrating better improvements than eugonadals.
- Few studies provided weak evidence of improvements of hypogonadism-related sexual symptoms such as erectile dysfunction, hypoactive sexual drive, and premature ejaculation.
- Most studies addressing varicocele-hypogonadism relationship have primarily focused on infertile men, with lacking evidence among fertile men. Thus, proposing varicocelectomy to treat hypogonadism among fertile men should not be considered as a standard of care and should be only contemplated on an experimental basis.

Introduction

A varicocele is defined as an aberrant dilatation and tortuosity of the pampiniform plexus of veins draining the testis [1]. It is a common condition with a prevalence of 15% in general male population. Varicocele is the most common cause of male infertility, representing 19–41% of men with primary infertility and 45–81% of men with secondary infertility [2, 3]. Although clinical (palpable) varicoceles are more commonly noted on the left side, the prevalence of bilateral varicoceles ranges from 30% to 80% [4]. Varicoceles are likely to have genetic predisposition. In a study by Raman and colleagues, varicoceles were observed in 56.5% of first-degree relatives of patients with varicoceles, compared to 6.8% of controls [5]. However, the genetic mechanisms and inheritance patterns remain to be elucidated.

T. A.-A. A.-M. Hamoda (✉)
Department of Urology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

Department of Urology, Faculty of Medicine, Minia University, El-Minia, Egypt
e-mail: ahamodh@kau.edu.sa

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In the majority of men, varicoceles are asymptomatic and have a minor impact on testicular functions. However, some men may present with infertility, testicular pain, testicular hypotrophy, and/or scrotal mass, which represent the usual indications for varicocelectomy [6, 7].

The linkage between varicocele and impaired spermatogenesis via effects on Sertoli and germ cells has traditionally been recognized [3, 6, 7]. Additionally, repair of clinical varicocele has been documented in high-quality studies, including randomized clinical trials and meta-analyses, to improve semen quality and pregnancy rates [8–12]. On the other hand, there is still growing evidence that varicocele presents a global testicular insult, and Leydig cell dysfunction is currently recognized as a ramification of varicocele which could be reversed with varicocelectomy [13, 14]. The pathophysiology of varicocele-related Leydig cell dysfunction and impaired testosterone biosynthesis is less clearly understood and remains an area of ongoing research. Nevertheless, several pathophysiological mechanisms have been proposed, likely indicating a multifactorial process [15].

In this chapter, we outline (1) the proposed pathophysiological mechanisms of varicocele with emphasis on varicocele-induced Leydig cell dysfunction, (2) the current evidence associating varicocele to diminished androgen production, (3) the effects of surgical repair of varicocele on hypogonadism, and finally (4) the influence of varicocelectomy on clinical (symptomatic) hypogonadism.

Pathophysiology of Varicocele-Mediated Testicular Dysfunctions

Impact on Germ Cell and Sertoli Cell

The theory of testicular hyperthermia (increased testicular temperature) due to poor venous drainage has traditionally prevailed explaining the detrimental effects of varicocele on germ cell, Sertoli cell, and Leydig cell functions [15, 16]. Normally, the scrotal temperature is kept 1–2 °C lower than core body temperature by a thin scrotal wall lacking subcutaneous fat, and a counter-current heat exchange system of the pampiniform plexus of veins, allowing arterial blood to be cooled before entering the testis [16]. Several animal and human studies have reported and linked testicular hyperthermia and heat stress in men with varicoceles to impaired spermatogenesis and infertility [17–21].

Further theories describing the detrimental effects of varicocele include (1) oxidative stress with excess seminal reactive oxygen species (ROS) and diminished seminal plasma antioxidant activity, (2) increased sperm DNA fragmentation, (3) decreased testicular DNA polymerase activity, (4) increased testicular cell apoptosis, (5) Sertoli cell dysfunction, (6) Leydig cell dysfunction with diminished testosterone biosynthesis, (7) testicular hypoxia, (8) impaired venous drainage of gonadotoxins, (9) reflux of renal and adrenal metabolites to testicular venous blood, and (10) formation of anti-sperm antibodies. While each of these theories has some evidentiary support, none of them can fully explain all the effects of varicocele, and a multifactorial process may be operational [15, 22–27].

Further details on the pathophysiological impact of varicocele and its repair on Sertoli cell, germ cell, spermatogenesis, sperm ultrastructure, and sperm functions are provided elsewhere in this book.

Impact on Leydig Cell

Adverse effects of varicocele on Leydig cell have also been reported, with consequential impaired testosterone biosynthesis and hypogonadism [7, 13–15]. Several in vitro, animal, human, and clinical studies have described Leydig cell ultrastructural and functional alterations linked to varicoceles. The proposed theories describing how varicocele may deleteriously impact Leydig cell are similar to those explaining varicocele-related infertility. It has been suggested that the previously mentioned mechanisms of hyperthermia and heat stress, oxidative stress, hypoxia, accumulation of gonadotoxins, and reflux of

renal/adrenal toxins can also affect Leydig cells in a similar fashion to Sertoli and germ cells [7].

Ando and his group have observed a tendency of diminished testosterone synthesis in varicocele men compared to control men. To examine for potential enzymatic block or dysfunction during steroidogenesis in men with varicocele, they have utilized an expanded assay of testosterone biosynthesis pathway before and after GnRH stimulation. They have reported a deficiency of 17,20-lyase activity, excess 17-hydroxyprogesterone, and a markedly elevated 17-hydroxyprogesterone/testosterone ratio in the varicocele group. Their findings imply a possible enzymatic impairment at the level of 17,20-lyase and, to a lesser extent, 17a-hydroxylase, which were proposed to be particularly sensitive to hyperthermia [28, 29]. Scholler et al. have also reported a blunted testosterone response in varicocele men compared to normal control over several time points after administration of large hCG bolus [30]. Sirvent and co-workers [31] have examined the testicular biopsies of a group of 31 varicocele patients. They have demonstrated hyperplasia of Leydig cells with cytoplasmic vacuolization and atrophy and a decreased number of Leydig cells stained for testosterone. It is notable that all examined patients demonstrated normal serum testosterone, FSH, and LH levels. These observations endorse the assumption that hyperplasia of Leydig cells may compensate for the atrophy and other changes to preserve serum testosterone normality. Similar findings were also reported by Francavilla et al. [32] who observed Leydig cell hyperplasia, correlating with the severity of concomitant oligozoospermia, in testicular biopsies from varicocele men. The ability of Leydig cells, biopsied from men presenting with concomitant varicocele and oligozoospermia, to produce testosterone was tested in vitro by Weiss and co-investigators [33]. They observed deficient testosterone synthetic activity, despite the men having normal serum testosterone and LH levels. The authors concluded that men with varicocele and oligozoospermia may have lower intratesticular testosterone levels that drive hypospermatogenesis. Remarkably, Leydig cell ultrastructural and functional alterations were identical in both testicles even with unilateral varicocele, endorsing the concept that a unilateral varicocele results in bilateral testicular changes.

Animal research has additionally elucidated the pathophysiology of varicocele-induced testicular insult and androgen production impairment [34–40]. The strength of the experimental animal studies is the use of control groups, which allows providing robust evidence. In the early and remarkable study by Rajfer and colleagues [34], who induced unilateral left varicocele rat model, the investigators' observations echoed the findings reported in humans [28–33]. Two weeks after induction of varicocele, they observed significant reduction of the intratesticular testosterone within the left testis, compared to control animals. They demonstrated a progressive and continuous decline of intratesticular testosterone with longer varicocele durations. They have also reported significantly diminished activities of the 17,20-lyase and 17a-hydroxylase enzymes in the testicles of varicocele rats. Notably, the contralateral testis paralleled the left testis in both varicocele and control animals, confirming that a unilateral varicocele insults both testicles [34]. Other investigators have observed significant reduction of intratesticular testosterone along with significant decrease of messenger RNA for the steroidogenic acute regulatory protein (StAR) [39]. Both expression and activity of StAR were found to be particularly vulnerable to oxidative stress, while the effect reversed following removal of the insult [39, 40].

The interplay between varicocele-mediated Sertoli cell dysfunction, Leydig cell dysfunction, and impaired spermatogenesis is still elusive. Several authors have linked diminished testosterone production to impaired spermatogenesis and infertility; as a cause-effect relationship [7, 33, 41–44]. Other investigators, however, deny this relationship and assume that both impaired testosterone production and impaired spermatogenesis are independent events sharing a common root-cause [21, 45–47]. Yet, it seems that both assumptions are interplaying.

The previous findings of Leydig cell ultrastructural and functional time-dependent alterations together with the proposed diminished

intratesticular testosterone, despite having normal serum testosterone [28–34], have driven some authors [47–49] to suggest varicocele repair even in men with normal semen parameters as an option to halt future deterioration of Leydig cell and hypogonadism particularly in young adults.

Varicocele and Hypogonadism: Clinical Research

Early clinical studies examining serum testosterone concentrations in varicocele men have demonstrated contradictory results, with some studies reporting androgen deficiency [28, 50, 51], while others refuted the concept that varicoceles result in decreased testosterone synthesis [52, 53]. Most of the initial studies were limited by being retrospective, underpowered, or addressing testosterone changes as secondary outcomes. The association between varicocele and androgen deficiency was addressed in a large study by the World Health Organization (WHO) reporting on 9034 men presenting for fertility issues [3]. The authors have observed that varicocele men >30 years old had significantly lower serum testosterone concentrations compared to varicocele men younger than 30 years, a trend not observed in men without varicocele. The later finding denotes a progressive duration-dependent negative impact of varicocele on Leydig cell function. In a 1995 retrospective study, Su et al. [54] have reported significant increase of mean serum testosterone from 319 ng/dL to 409 ng/dL following varicocele repair in 53 infertile men. Notably, their report was one of the earliest emphasizing a significant inverse correlation between preoperative testosterone levels and change in postoperative testosterone, as they have noted that men with lowest preoperative testosterone levels demonstrated the highest improvements after surgery. These authors also reported significant positive correlation between testicular firmness and improvement of serum testosterone, as men with at least one firm testicle experienced better improvement of testosterone compared to men with bilateral soft testicles.

More recent well-designed adequately powered studies, however, have better highlighted the deleterious effects of varicocele on serum testosterone levels and have provided better quality evidence. Tanrikut and co-authors have implicated varicocele as a risk factor for androgen deficiency, as they reported statistically significant lower serum testosterone levels in varicocele men compared to no-varicocele men (412.2 ng/dL vs 462.6 ng/dL, respectively) [55]. The same group [56] have later compared a cohort of 325 infertile men with varicocele who had undergone varicocelectomy to 510 men who presented for vasectomy reversal surgery with proven previous fertility and no palpable varicocele. The varicocele group exhibited significantly lower baseline mean serum testosterone levels than the comparison men (416 ng/dL vs 469 ng/dL), with the difference remaining statistically significant after controlling for age. Out of 200 men with documented pre- and post-varicocelectomy serum testosterone levels, most patients demonstrated improved serum testosterone concentrations, with a mean postoperative testosterone increase of 96 ng/dL (358 ng/dL vs 454 ng/dL, respectively). Notably, among the preoperatively hypogonadal patients (testosterone levels <300 ng/dL), 79% exhibited postoperative eugonadal status (testosterone levels >300 ng/dL) [56]. Several other contemporary studies have also boosted the finding of improved serum testosterone levels following repair of varicocele [57–60], which is particularly evident in men with baseline hypogonadism. Hsiao et al., using a serum testosterone cutoff of ≤400 ng/dL, have retrospectively classified 272 men prior to varicocelectomy into biochemical hypogonadal or eugonadal men. Similarly, the hypogonadal men with lower baseline serum testosterone responded far better after varicocelectomy compared to eugonadal men [57]. Other researchers have observed similar outcomes and confirmed that the improvements of androgen levels after repair of varicocele were found to be inversely correlating to the baseline serum testosterone levels with higher response in hypogonadal men compared to eugonadals

[54, 56–60] and were positively correlating with testicular firmness [54]. However, testosterone changes were reported to be independent of age of patient [57], laterality of varicocele, or clinical grade of varicocele [58].

To test the hypotheses that varicocele insults androgen production and varicocele repair improves serum testosterone levels, we have conducted a prospective controlled nonrandomized adequately powered study totaling 165 adult men distributed over four groups [60]. Group 1 involved 66 clinical varicocele-infertile treated men; group 2 included 33 clinical varicocele-infertile untreated control men; group 3 included 33 clinical varicocele-fertile untreated control men; and group 4 encompassed 33 fertile men without varicocele (normal control). Only men in group 1 received microsurgical subinguinal varicocelectomy, while other varicocele men were observed. Varicocele groups were further subgrouped into baseline hypogonadals (testosterone <300 ng/dL) or eugonadals (testosterone ≥300 ng/dL). The main outcomes were the cross-sectional between-group baseline testosterone differences; and the longitudinal within-group testosterone changes at 6- and 12-month points of time in varicocele men. The means of baseline serum testosterone in the four groups were 347.4, 339.7, 396.6, and 504.8 ng/dL, respectively. At baseline, all varicocele groups' testosterone levels were matching, whereas they were significantly lower than those of the normal-control group. Only the subset of men with pre-existing biochemical hypogonadism (<300 ng/dL) experienced significant increase of testosterone concentrations 6 months following varicocele repair (mean testosterone level increase of 93.7 ng/dL), while the eugonadal men demonstrated insignificant increase (8.6 ng/dL). These improvements were maintained at 12-month follow-up. In spite of these remarkable improvements, the varicocelectomy men did not attain increased testosterone levels comparable to those of normal-control fertile men with no varicocele. Contrary to varicocelectomy group, other non-treated varicocele groups demonstrated insignificant testosterone changes [60].

As our study [60] has bolstered the findings of the previous reports [54–59], subsequent prospective studies have also complemented our findings [42, 61]. Ahmed et al. [61] prospectively studied a cohort of 73 varicocele men up to 6 months after varicocelectomy, to compare them to a matched control group of 56 men who were observed only. The authors reported significant mean testosterone concentration increase from 332 to 358 ng/dL in the varicocelectomy group, while the observed group showed nonsignificant changes.

Recent meta-analyses studies have also endorsed the evidence coupling varicocelectomy to improved testosterone concentrations and have favored varicocelectomy [13, 14]. Li et al. [13] carried out a systematic review and meta-analysis of combined data of nine studies totaling 814 men with varicocele and infertility, to report a significant summative mean testosterone increase of 97.5 ng/dL after varicocelectomy. Later, Chen and colleagues [14] conducted a meta-analysis of clinical trials and retrospective studies comparing pre- and post-varicocelectomy serum testosterone, to combine eight studies and 712 subfertile patients. The pooled analysis of seven studies revealed significantly improved mean serum testosterone after surgery by 34.3 ng/dL, compared to the preoperative values. In further subgroup analysis, the hypogonadal treated men demonstrated more remarkable and significant improvement of testosterone levels by 123 ng/dL, while the eugonadal treated men and the untreated controls experienced nonsignificant changes.

Despite the compelling evidence of linkage of varicocele to impaired androgen production and the efficacy of surgical repair in reversing this deleterious process, some reports [62–64] in the more recent literature—similar to the earlier literature [52, 53]—still deny the association between varicocele and hypogonadism and contradicted any significant improvement of androgen production following varicocele repair. Yet, most of these studies were flawed by several weaknesses and limitations such as a retrospective design, limited number of subjects, selection bias, and testing for testosterone as a secondary outcome with undermined statistical

power. More important, the studies disputing any significant change of androgen after varicocelectomy mostly recruited eugonadal men at baseline and the investigators did not conduct a distinct subgroup analysis of hypogonadal men [62–64].

Varicocele and Clinical (Symptomatic) Hypogonadism

Although testosterone plays a key role in the male sexual functions, only very few researchers have studied the association between varicocele/varicocelectomy and hypogonadism symptoms, such as erectile dysfunction (ED), hypoactive sexual drive, as well as premature ejaculation (PE). Younes has published a report in 2003 referring to an improvement of sexual activity in 50%–75% of impotent men after varicocelectomy [65]. In a prospective study [66] by Zohdy et al., 141 infertile patients with palpable varicocele were treated using either varicocelectomy or assisted reproductive technologies (ART). They reported significantly improved serum testosterone levels 6 months after varicocelectomy (from mean 379.1 to 450.1 ng/dL). The improvement in testosterone was particularly pronounced in men with pre-existing hypogonadism (from mean 219.4 to 358.2 ng/dL). The authors noted attaining normalization of serum testosterone after surgery in 75.5% of baseline hypogonadal men. No similar changes were seen in the ART group of men. Regarding sexual functions, a total of 76 men (53.9%) reported ED at baseline with International Index of Erectile Function (IIEF-5) score less than 21 points, while hypoactive sexual desire and premature ejaculation were observed in 95/141 (67.4%) and 31/141 (22%) men, respectively. Their study also revealed an improved erectile function after varicocelectomy. Among the men reporting pre-existing ED, 12.6% demonstrated improved IIEF-5 scoring above 21 points after varicocelectomy. The improved erectile function was also more evident among baseline hypogonadals with ED, where 30.6% of them reported improvement with more than 21 points on IIEF-5 score after surgery. Conversely, men in the ART group did not exhibit such improvements in IIEF-5 scores. Notably, both improvements of testosterone and IIEF-5 after varicocelectomy were found to be positively and significantly correlating ($r = 0.629$, $p < 0.0001$). In a very similar study [67], Srini and Veerachari prospectively reported on 200 infertile men with varicocele who were also treated with either varicocelectomy or ART. Hypoactive sexual drive, premature ejaculation, and prevalence of ED were noted at baseline in 72%, 15.5%, and 53.9% of all patients, respectively, which the authors assumed to be attributable to low testosterone linked to varicocele. The investigators observed significant increase in serum testosterone among varicocelectomy men with 78% of men becoming eugonadal 6 months after varicocelectomy, in contrast to 16% of the ART men. The authors have also noted improved erectile function as well as decreased ED prevalence from 44% to 31% among men undergoing varicocelectomy, as compared to a slightly increased ED prevalence among the ART men. Additionally, Najari and colleagues [68] in 2016 published a retrospective study evaluating the effects of microsurgical varicocelectomy on serum testosterone levels and sexual functions as measured by the Male Sexual Health Questionnaire (MSHQ). They reported significant improvements in the serum testosterone levels as well as total MSHQ score, MSHQ erectile function domain, and MSHQ ejaculatory function domain after varicocelectomy.

Premature ejaculation and varicocele linkage was addressed in 2009 by Lotti and colleagues [69], who have noted significant association of the two conditions (PE in 29.2% vs 24.9% of men with or without varicocele, respectively). Ahmed and colleagues [61] have later prospectively studied the effect of microsurgical subinguinal varicocelectomy on PE, erectile function, and serum testosterone in 73 patients with clinical varicocele associated with premature ejaculation, compared to nonrandomized 56 comparable control men having no surgery. The examined outcomes included mean changes in premature ejaculation diagnostic tool (PEDT), IIEF-5 score, and total serum testosterone. The treated men

demonstrated improved ejaculatory function with significant drop of mean PEDT score from 15.6 to 11.4. PE improved in 41.1% of the treatment group compared to 5.3% of the control group. They also noted significant improvements in IIEF-5 scores and testosterone levels among treated men, but not in control men. The authors concluded that varicocelectomy is related to improvements of premature ejaculation, erectile function, and testicular hormonal function. Yet, investigators did not clearly describe the fertility status of the studied patients, nor the inclusion criteria as they stated including "patients with idiopathic varicocele, who visited the urology and andrology clinics for different genitourinary complaints."

Nevertheless, these studies were limited by a retrospective or nonrandomized controlled design, or were based on a small sample size, and thus yielding insufficient strength of evidence. Particularly among infertile men, sexual functions may be affected by social constrains, anxiety, or other psychological factors. Hence, a placebo effect of varicocelectomy might confound the subjective symptomatic outcomes of surgery and needs to be better identified [70]. Therefore, the hypothesis of improvement of hypogonadism symptoms and sexual symptoms after surgical repair of varicocele is still lacking good evidence and still requires further testing with high-quality properly designed, properly conducted, prospective large-scale randomized controlled studies. Yet, until a robust evidence becomes in hand, the patients can be still carefully counselled about the potential to improve serum testosterone and sexual functions by varicocelectomy.

Conclusion

Over several decades, numerous high-quality studies, including many randomized controlled trials and meta-analyses, have linked varicocele to infertility and have endorsed a beneficial effect of varicocelectomy on semen quality among infertile men, to provide a high-level evidence favoring treatment. Nevertheless, not as much of studies addressing the linkage between varicocele/varicocelectomy and androgen production among infertile men are existing in the contemporary literature, although the body of evidence of such linkage is growing stronger and favoring varicocelectomy. Unfortunately, there are no known studies on the long-term durability of improved androgen production after varicocele repair, and thus we are raring to see such long-term outcomes.

While the preponderance of studies addressing varicocele-hypogonadism relationship have primarily focused on infertile men populations, there is a noticeable paucity of studies addressing the linkage between varicocele/varicocelectomy and androgen production among fertile men. To further cloud the scene, it is difficult to extrapolate the results of the studies on infertile men with varicocele and hypogonadism to non-infertile men. The last concerns generated a great deal of controversy and still a matter of intense debate among the medical community. Robust evidence addressing the association between varicocele and hypogonadism among fertile men is still lacking. Thus—at the current time—proposing varicocelectomy as a treatment of biochemical or symptomatic hypogonadism among fertile men should not be considered as a standard of care in the routine practice and should be only contemplated on an experimental basis.

Review Criteria

A search of studies examining the relationship between varicocele and hypogonadism was performed using search engines such as PubMed, ScienceDirect, OVID, MEDLINE, and Google Scholar. The overall search strategy was based on the following keywords: "androgen," "hypogonadism," "infertility," "Leydig cell," "testicle," "testosterone," "varicocele," "varicocelectomy," "sexual dysfunction," and "spermatogenesis."

Multiple Choice Questions and Answers

1. The reported prevalence of hypogonadism among men with varicocele is:
   (a) 15%
   (b) 19–41%
   (c) 56.5%
   (d) **None of the above**
2. The most probable theory explaining the pathophysiology of varicocele impact on Leydig cell is:
   (a) Heat stress
   (b) Oxidative stress
   (c) Impaired drainage of gonadotoxins
   (d) **A multifactorial theory**
3. Robust evidence supports the linkage of varicocele to:
   (a) **Infertility**
   (b) Hypogonadism
   (c) Erectile dysfunction
   (d) All of the above
4. The current evidence supports repair of clinical varicocele in:
   (a) Fertile men with biochemical hypogonadism
   (b) **Infertile men with impaired semen parameters**
   (c) Infertile men with erectile dysfunction
   (d) Any of the above
5. Changes in serum testosterone levels following varicocelectomy were reported to be correlating with:
   (a) Age of patient
   (b) Grade of varicocele
   (c) **Baseline serum testosterone**
   (d) Testicular size

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39 What Should Be the Ideal Control Group in Clinical Trials Investigating the Role of Varicocele and Its Treatment on Fertility Outcomes?

Renata Cristina de Carvalho and Renato Fraietta

Key Points

- Clinical trials are studies that indicate the effectiveness of an intervention over a particular condition. A control group is essential in this study design in order to accurately compare results.
- Individuals enrolled in a clinical trial have similar characteristics and can be allocated in the control group or the study group.
- In varicocele treatment studies, the intervention effectiveness over fertility potential can only be determined in presence of a control group, which is necessary for comparison and evaluation of any possible improvement.
- There are three main approaches in varicocele intervention studies: comparison between treated and untreated individuals, different treatment methods, and interventions at different points of time.
- In summary, the ideal control group is the one that gives support to validate a desired endpoint.

Introduction

Varicocele is a condition capable of affecting the male fertility potential by altering seminal parameters as concentration, motility, and morphology of sperm as well as well as various sperm functions such as levels of DNA fragmentation and mitochondrial activity [1–3].

According to the American Society for Reproductive Medicine guideline [4], the altered seminal parameters may justify varicocele treatment. In addition, other aspects indicating treatment can include palpable varicocele at diagnosis, couple having proven infertility, and/or female fertility being normal. To determine the real effectiveness of treatment, however, clinical trials are necessary.

A clinical trial is defined as a prospective study in which an individual undergoes one or more interventions to evaluate their effects on health-related outcomes [5]. In order to perform such analysis, a comparator of results is necessary, meaning that in order to know if the intervention performed is satisfactory, it is necessary to compare the findings with individuals who did not perform the same intervention, individuals that compose the so-called control group [6].

R. C. de Carvalho · R. Fraietta (✉)
Department of Surgery, Division of Urology, Sao Paulo Federal University, Sao Paulo, SP, Brazil

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Purpose of Control Groups

The purpose of a control group in a clinical trial is to indicate what happens to an individual when he/she does not receive the intervention under study or when he/she receives a treatment different from the one already established in the literature as the gold standard for a given condition. The control group is pivotal to a research protocol as in most of the cases the results cannot be predicted. The individuals that compose this group are selected from the same population frame as the study group that will receive the evaluated intervention, in a way that both groups will have similar characteristics, highlighting only the effects of the applied intervention [7].

Types of Controls

The control group profile is based on the purpose of the clinical trial. It is necessary to define the type of intervention under study, and from there, the control individuals will belong to one of the four categories:

1. Placebo control group
2. Control group without intervention
3. Control group different regime
4. Control group alternative intervention

The placebo group is widely used for the development of new drugs and works by inducing a classic approach: the individual receives a treatment that does not contain the drug that is under study. In studies using the control group without intervention, individuals are randomly selected to not receive the intervention under study. When choosing to use a different regime as an intervention, individuals from the control group will receive a different intervention or treatment than the study group; a classic example of this type of control is the varied dosages of a certain drug. Finally, the control group receiving an alternative intervention is the one receiving a gold standard treatment or intervention already established for a given condition, while the study group will receive a new treatment [7].

Types of Clinical Trials and Its Controls

Not all varicocele requires treatment. To substantiate a choice of the best treatment approach, it is necessary to test and confirm if the intervention has brought benefit to an affected individual, mainly related to changes in the male fertility potential that is a weight justification for intervention of such condition. Thus, a clinical trial with comparison of the results with the selected control group becomes an important investigative method to define the best way of improvement of fertility potential.

Among the different study designs that investigate the treatment of varicocele and its benefit for male fertility, three approaches are highlighted: comparison between treated and untreated individuals, comparison of different treatment techniques, and comparison of interventions in different points of time. All these studies require a control group necessary for results comparison.

Treated and Not Treated

In this study design, the main purpose is to verify whether the treatment of varicocele will bring improvements to the male fertility potential answering the question: Does varicocele treatment improve seminal parameters and quality? In this case, ideal control individuals should be diagnosed with dilatation of the pampiniform venous plexus but receive no treatment. This design was used by Yamamoto et al., on a study with the aims to determine if treatment of subclinical varicocele improves fertility and/or seminal parameters – participants were randomized into two groups: one undergoing surgery (high ligation of the internal spermatic vein) and a second with individuals who received no treatment acting as a control group. The study concluded that surgery has an effect on spermatogenesis but does not increase pregnancy rates [8]. Grasso et al. also used similar design to verify the effects of repair of grade 1 varicocele on seminal quality using a control group composed of individuals who were diag-

nosed with varicocele but received no treatment. This study, however, has found that treatment does not bring benefits to seminal quality [9].

Further investigations on male fertility potential can also be designed and still make use of control groups. García-Peiró et al. investigated the level of sperm DNA fragmentation in both men with subclinical and clinical varicocele after varicocelectomy. In this approach, as we have two study focus groups (different degrees of varicocele), two control groups are created: one with subclinical varicocele-diagnosed individuals and another with clinical varicocele-diagnosed individuals – both without receiving the proposed treatment [10].

Comparison Between Two Therapies

In clinical trials that aim to analyze the benefits of a new treatment for a particular condition, the control group is composed of individuals who will receive the gold standard treatment already well established in the field. A classic example regarding varicocele is a study performed by Kucuk et al. that evaluated the effect of acupuncture on pregnancy rates and seminal parameters of men diagnosed with varicocele. Control group in this case were men who went through the gold standard treatment, varicocelectomy. The study concluded that acupuncture is an effective treatment for men with varicocele, primary infertility, and altered semen analysis [11].

This design of clinical trials can also be useful to improve techniques already established as effective treatments for varicocele. This is evidenced by Wang et al. with a study aiming to evaluate if the LESS laparoscopic single-channel varicocele ligation technique is more effective than the conventional transperitoneal laparoscopic varicocele ligation technique (control group). The authors did not obtain satisfactory results in the seminal analysis when comparing the two techniques but concluded the LESS technique under study decreases postoperative pain [12].

There are some study designs that do not explicitly require a control group – it happens when several treatment techniques are established for a certain condition, but the researcher wants to find out which one is the most effective to a certain parameter. In the context of varicocele and male infertility, it is important to determine which treatment method is the most effective in improving the male fertile potential as shown in studies by Sun et al., Barbalias et al., Pajovic et al., and Yavetz et al. [13–16].

Finally, it is possible to study if certain medications improve the seminal quality of men with varicocele – in this case it is important to create a placebo control group, as previously described in the section "Types of Controls." In the study by Cavallini et al. which aimed to verify if the use of an anti-inflammatory drug and varicocelectomy are adequate treatments for oligoastenozoospermia of men with varicocele, a placebo control group (a glycerin capsule with similar shape and forms to the studied drug) was included, and the study concluded that the anti-inflammatory drug proved to be effective for varicocele grade III while surgery should be indicated for other varicocele grades [17].

Treated Within Different Occasions

In clinical trials it is also feasible to study different points of time of intervention/treatment of the studied condition. In the case of varicocele, the main focus of this type of study is to evaluate the best moment of treatment (adolescence or adulthood) and the influence of each point in time in the fertile potential outcome. In order to have a measure of comparison, the control group in this design may be the group in which the effects of the treatment are better known [18, 19].

Furthermore, Madgar et al. promoted a design with two randomized groups: the first group composed of men who underwent surgical repair of varicocele only 1 year after the participants' recruitment and the second group composed of men who underwent treatment at the moment they were enrolled in the research. Group 1 at baseline was used as control comparing the findings before and after surgery (type of study the section "Treated and Not Treated") and at the endpoint verifying the behavior of varicocele in different points of time of treatments [20].

Control Groups in Basic Research

Basic research aims to understand the influence of a condition on a particular aspect. In the case of varicocele and male fertility, the basic research goal is to elucidate the mechanisms by which this condition changes the male fertility potential through basic seminal analysis, spermatozoon functional aspects evaluation, and/or semen molecular analysis. In this situation, the selected control group should be different from those in clinical trials – the control group should be composed of men without a diagnosis of varicocele who also have no signals or symptoms that could modify their fertility potential. Therefore, the study will compare diseased participants and related outcomes with non-diseased individuals who will provide parameters of an expected normal seminal sample.

Basically, when conducting this type of research, we will define our inclusion and exclusion criteria for the two selected groups: study group with individuals diagnosed with varicocele and control group with a variable profile according to the hypothesis and purpose of the study. A study by Bertolla et al., for example, recruited young males (15–17 years old) with and without diagnosis of varicocele grade II or III for the study and control groups, respectively [1]. Another possibility is observed in the studies by Zylbersztejn et al., Belardin et al., and Del Giudice et al. which recruited two study groups (both with young men diagnosed with varicocele, but one presenting with seminal analysis impairment and one with normal seminal analysis) and one control group (young males without varicocele and normal seminal analysis) [21–23]. Another design was chosen by Mori et al. who selected three groups: the first with adolescents diagnosed with grade III varicocele, the second with adolescents diagnosed with grade II varicocele, and the last as the control group composed of adolescents without varicocele or varicocele grade I – this design was selected with the purpose to evaluate the impact on seminal quality according to the varicocele severity [3]. Further research designs may also be performed with adults, but the inclusion criteria of the control group might remain the same: men not diagnosed with varicocele, as done by Blumer et al. who aimed to investigate the effect of varicocele on sperm function and lipid peroxidation [24]. Besides inclusion criteria, it is also important to determine exclusion criteria in order to remove bias when analyzing the effects of varicocele on male fertility – the mentioned studies used strict exclusion criteria as history of systemic diseases, endocrine alterations, urological surgery, and inflammatory processes, among others.

Sample Size and Statistical Considerations

A clinical trial, as any other research, should start with a protocol to be followed including the points: study design, purpose, data collection methodology, profile of the selected population, how many individuals should be recruited in order to obtain meaningful results (sample size calculation), and finally the adequate statistical method of choice [25].

The main goal of a sample size calculation is to determine the amount of individuals that should be evaluated in order to detect a clinically relevant effect of the intervention under study and/or to determine the intervention safety and effectiveness [26, 27]. The sample size calculation is pivotal on a study design, and if not well conducted and justified, the study is considered to be of low quality with non-relevant results [28].

Despite the existence of pre-established equations to determine the ideal sample size of each study, it is necessary to understand that the calculation considers four main points:

1. Level of significance: ideal values are those greater than or equal to 5% – the smaller the level of significance, the larger is the calculated sample size.
2. Statistical power: this indicates the sensitivity of a test, ideally greater than or equal to 80% – the larger the sample size, the greater is the statistical power.
3. Clinically significant difference: this corresponds to the minimum difference found

between study groups that may be considered clinically relevant – the larger the sample size, the smaller is the clinically significant difference.
4. Variability: this is data corresponding to the variability of study population in previous studies [25, 29, 30].

Failure to consider these points may cause certain issues for the clinical trial. A poorly justified sample size calculation leads to under- or overestimation of the sample. Underestimating a sample size (selecting a number of participants lower than necessary) may result in non-statistically significant outcomes when they should be in fact significant. Overestimating a sample size (selecting a number of participants greater than necessary) may achieve the expected statistical outcome however exposing more individuals than necessary to an intervention that may lead to ethical issues. These sample size errors may cause erroneously conclusions rejecting effective interventions (type II error) or approving ineffective treatments (type I error) [25].

As determined by the Consolidated Standards of Reporting Trials (CONSORT) statement, the sample size calculation of each study should be reported and justified in the clinical trial protocol [31].

Main Outcome Measures

There are several analyses to understand the relationship between varicocele and treatment over fertility results.

Starting from basic approaches, conventional seminal analysis (spermogram) [32] is the most applied protocol in studies seeking to understand the effects of varicocele and its repair over male fertile potential. A meta-analysis by Agarwal et al. investigated the effect of varicocele on the seminal parameters of infertile men including only published studies that contained a group with infertile men with varicocele and a control group formed by fertile men or classified as normozoospermic without varicocele – the conclusion was that varicocele decreases sperm concentration, motility, and morphology [33]. Regarding varicocele repair, Al Bakri et al. found an improvement on seminal parameters 3 months after surgery – in this study the control group was each man's own sample before the treatment [34].

With the progress of knowledge, it became possible to investigate other seminal aspects, further studying the spermatozoon and its detailed characteristics in the presence of varicocele and after the treatment. A meta-analysis by Wang et al. found that men with varicocele have greater damage in sperm DNA when compared to control individuals and that varicocelectomy can improve this condition [35]. Similar results were obtained by Lacerda et al., who also verified an improvement in the DNA fragmentation of adolescents after varicocele correction surgery and also observed an increase in mitochondrial sperm activity [36].

In addition, it has become important to understand the action of varicocele on the molecular mechanisms of semen, and for this purpose studies have included, for example, plasma protein analysis of men diagnosed with this condition. Camargo et al. studied seminal samples of adults with varicocele before and after surgery and verified the presence of exclusive proteins in both the preoperative group (proteins related to the metabolism and regulation of nitric oxide) and the postoperative group (proteins linked to the cellular response to reactive oxygen species, glyconeogenesis, and protein stabilization) [37]. Similarly, Lima et al. verified the presence of HSPA2 gene mRNA (molecular chaperone that supports the folding and transport of proteins) in spermatozoa of adolescents with (study group) and without (control group) varicocele and concluded the expression is down-regulated in adolescents with varicocele and oligozoospermia [38].

Finally, after all these analysis, it is important to understand how varicocele interferes with fertility itself, an aspect assessed through achievement of pregnancy. A meta-analysis by Kim et al. verified the impact of varicocele repair on pregnancy rates including only studies comparing men who were treated by surgery with untreated controls and observed that varicocele repair may lead to a significant improvement in pregnancy rates [39].

Difficulties in Conducting Varicocele Studies

The prevalence of varicocele in the population is 15%, but when we analyze the fertility itself, 40% of men with primary infertility and 80% of men with secondary infertility are carriers of this condition [40]. Recruiting patients to study the condition and possible treatments is relatively simple, especially when recruitment occurs at infertility clinics. The major challenge for these studies is to recruit individuals for the control group in both clinical trials (affected individuals who will not receive any treatment benefit or at least not the best-known intervention) and basic research (men without any abnormalities that can influence the results).

In order to reach the ideal control group, great effort is essential to investigate the physiological characteristics of each individual so as to correctly classify him as a suitable control or not. Exhaustive search for participants is necessary, because the greater the number of people, the greater the probability of finding the ideal control. In addition, the objective of the study should be well evaluated in order to recruit the best comparison group and the best control group (untreated, treated with standard therapy or at the best time).

Conclusion

The ideal control group in clinical trials investigating the role of varicocele and its treatment on fertility outcomes depends on the purpose of the study taken into consideration. If the study aims to evaluate improvements in male fertility potential, the ideal control group is the one with varicocele but receives no treatment. If the objective is to analyze the benefits of a new therapy, the control group is composed of individuals who will receive the gold standard varicocelectomy. If the effect of treatment in different points in time is to be observed, the control group may be the group in which the effects of the treatment are better known. Studies aiming pregnancy outcomes based on varicocele treatment are more complex to design because of the influence of the female variables and its heterogeneity.

Review Criteria

For this chapter writing, PubMed database was used. The articles were selected by the following keywords: "varicocele," "male fertility," "clinical trial," "control group," "basic research," "statistics," and "sample size." The relevant references cited in the selected articles were also taken into account. All articles referenced were in English language. The publications used for this survey were not limited by period.

Multiple Choice Questions and Answers

1. What is the purpose of a control group on a clinical trial?
   (a) To increase sample size.
   (b) **To have a comparative parameter of the intervention.**
   (c) To not have a comparative parameter of the intervention.
   (d) A control group is not necessary and so there is no purpose.
2. What is the main characteristic of an individual from a control group on a clinical trial?
   (a) Derived from a different population than the study group.
   (b) Not affected by the studied disease.
   (c) Individuals go through the same intervention as the study group.
   (d) **Derived from the same population than the study group but not going through the same intervention.**
3. All the options below are approaches of a clinical trial evaluating varicocele treatment related to male fertility, except:
   (a) Treated and untreated groups
   (b) Comparison of two different techniques
   (c) Intervention in different points of time
   (d) **Groups treated with the same therapy in the same point of time**
4. What is the purpose of a clinical trial comparing two different therapies for varicocele?
   (a) **To analyze the benefits of a new treatment**

(b) To verify if the two studied treatments are effective
(c) To verify what is the best point in time for the treatment
(d) To analyze the varicocele effects on male fertility

5. What is the most performed test when evaluating varicocele and treatment effects over male fertility?
(a) Hormonal analysis
(b) Complete blood count
(c) **Conventional seminal analysis**
(d) Analysis of oxidative stress

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Part V

Varicocele Debates: Pro and Con

40 Should Sperm DNA Fragmentation Testing Be Used in Men with Varicocele?

Chak-Lam Cho

Key Points

- SDF testing emerges as an advanced sperm function test over the last few decades in view of the poor performance of semen analysis in differentiating fertile and infertile men with varicocele.
- The understanding of the central role of oxidative stress and SDF in the pathophysiology of varicocele-associated male infertility supports SDF testing as a potential biomarker.
- The implication of SDF in pregnancy outcomes, particularly natural conception, and embryo health is supported by current evidence.
- Presence of varicocele is associated with higher SDF and varicocele repair can effectively reduce SDF.
- Recent guidelines on clinical utility of SDF testing have been published. The application of SDF assays in men with varicocele potentially allows better selection of surgical candidates, earlier intervention to halt progressive testicular dysfunction, and more accurate prediction of postoperative treatment efficacy.

Introduction

Varicocele is one of the most controversial topics in urology. Despite the clear association between varicocele and male subfertility, the question why most men with varicocele have no apparent fertility issue remains unanswered. A better understanding of the pathophysiology of varicocele-associated male infertility is of paramount importance in better selection of appropriate candidates who will benefit from varicocele repair. By the same token, additional laboratory tests are also eagerly required in view of the poor predictive value of conventional semen analysis on male fertility potential and reproductive outcomes [1].

Over the last few decades, the recognition of sperm quality and the implication of sperm DNA on fertility was identified [2, 3]. Recently, the central role of oxidative stress in varicocele-associated male subfertility and its association with sperm DNA breaks was established [4]. Following the introduction of various sperm DNA fragmentation (SDF) assays in the 1980s and 1990s, the pace of research in the area accelerated. Although the routine use of SDF tests in male factor evaluation is generally not supported by professional societies currently [5], the potential role of SDF has been acknowledged in the latest American Society of Reproductive Medicine, American Urological Association, and European Association of

C.-L. Cho (✉)
S.H. Ho Urology Centre, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

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Urology guidelines on male infertility [5–7]. The clinical utility of SDF testing based on the current best evidence has been recently summarized by the Society for Translational Medicine [8]. The more extensive use of SDF tests among fertility specialists reported by a recent survey reflects increasing acceptance of SDF testing into clinical practice [9].

In this debate, the supporting reasons for why SDF testing should be used in men with varicocele will be presented.

Sperm DNA Fragmentation Is Associated with Fertility Potential

The clear relationship between SDF and natural conception is supported by good quality data using the excellent endpoint of time-to-pregnancy. The Danish First Pregnancy Planner study demonstrated a decrease in fecundability in association with high SDF in 250 Danish couples without previous knowledge of their fertility capability [10]. Similarly, in the Longitudinal Investigation of Fertility and the Environment (LIFE) study, the correlation between high SDF and low fecundability was again reported in 501 couples with no infertility history discontinuing contraception for the purpose of becoming pregnant [11]. A meta-analysis involving three studies and 616 couples concluded high SDF was associated with failure to achieve natural pregnancy with an unambiguous odds ratio of 7.01 (95% CI 3.68, 13.36) [12]. On the other hand, the less clear relationship between SDF and assisted reproductive outcomes represents the resistance in wider clinical application of SDF testing [13]. The phenomenon is particularly obvious in intracytoplasmic sperm injection (ICSI) when the natural selection process during fertilization is bypassed with technology [13]. However, emerging data illustrated the effect of SDF on in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) outcomes may be mediated by an increased risk of miscarriage [14]. Indeed, couples whose male partners had low SDF may achieve higher live birth rates after IVF and ICSI [15]. More importantly, SDF is a useful biomarker for embryo quality and embryo development [16, 17]. It has been also speculated that SDF might lead to a higher risk of congenital disabilities in the offspring [18].

Although SDF has been associated with abnormal conventional semen parameters [16], studies on the possible correlation between SDF and conventional semen parameters yielded ambiguous conclusions. On one hand, a negative association between SDF and morphologically normal spermatozoa has been reported. On the other hand, the fact that sperm with high SDF can have normal motility and morphology suggests additional prognostic value of the assessment [19]. In fact, the value of SDF as an independent attribute of semen quality has been recently supported [20]. There are often opinions opposing the clinical application of SDF testing in view of the less-than-perfect precision in discriminating fertile and infertile male. However, it should be noted that SDF results, similar to fertility potential, should be conceptualized in terms of probability rather than a bimodal parameter, and it should not be defined by a simple “yes” or “no.” The quest for a single magic test in the context of the complex human reproductive system is probably an oversimplification. The coexistence of multiple male and female factors in an infertile couple will likely necessitate comprehensive assessment by a panel of diagnostic tests. SDF should be considered as a unique member in the panel rather than the single gold standard. The fact is well illustrated in the LIFE study that factors, including semen parameters and age of infertile couple, influence time-to-pregnancy of first pregnancy planners in addition to SDF [11]. SDF test results reflect overall sperm quality to a certain extent and are complementary to semen analysis, but more significant and distinct than conventional semen parameters.

Presence of Varicocele Is Associated with High SDF

A number of studies have examined the association between SDF and varicocele. Fertile and infertile men with varicocele tend to have higher

SDF than controls, thus suggesting that varicocele itself is associated with DNA damage even when fertility has not been compromised.

Early observational studies demonstrated elevated SDF in infertile men with clinical varicocele compared to normozoospermic donors [21]. It was reported that SDF in infertile men with varicocele (35.7% +/− 18.3% by sperm chromatin dispersion test) was among the highest in patients attending infertility clinic for various etiologies [22]. The correlation between SDF and varicocele was further confirmed by a systematic review and meta-analysis. Sixteen case-control studies evaluating fertile and infertile men with and without varicocele were included, and SDF was higher in infertile patients with varicocele than those without. The same observation was also noted in fertile men [23]. The result illustrated the close relationship between high SDF and presence of clinical varicocele irrespective of fertility status. Another meta-analysis echoed by reporting a significantly higher SDF of 9.84% (95% CI 9.19, 10.49; $p < 0.00001$) in 240 patients with varicocele than 170 normal healthy controls without varicocele from seven studies [24].

Varicocele Repair Represents an Effective Treatment for High SDF

The lack of effective treatment to reduce SDF is one of the major hurdles to clinical utilization of SDF. The situation is overturned by recent studies revealing the success of varicocele repair in alleviation of high SDF in the majority of patients. Over 20 studies included more than 1000 treated patients were published in the last two decades focusing on the effect of varicocelectomy on SDF [25]. It is observed in an early study that 90% of the patients showed a significant decrease in SDF 3–6 months after varicocelectomy in 11 men with clinical varicocele [26]. Another retrospective cohort study also reported improvements in SDF in 78% of the treated patients [27]. A systematic review involving 511 patients from 3 retrospective and 9 prospective studies comparing men with clinical varicocele with a control group demonstrated a decrease in SDF after varicocele repair [23]. A meta-analysis also revealed a 3.37% (95% CI 2.65, 4.08; $p < 0.001$) reduction in SDF after varicocele repair [24]. A similar outcome was observed in adolescents who demonstrated increase in sperm DNA integrity after varicocele repair [28].

Moreover, the beneficial effect of varicocele repair in reducing SDF translates into increased pregnancy outcomes. A prospective study evaluated 49 men with palpable varicocele and oligozoospermia with at least 1-year history of infertility. In addition to a reduction in SDF at 3 months after varicocelectomy, 37% of the couples achieved a natural pregnancy and 24% achieved pregnancy with assisted reproductive techniques (ART) at 2 years after varicocelectomy. The post-varicocelectomy mean DNA fragmentation index (DFI) by sperm chromatin structure assay (SCSA) was significantly higher in couples who did not achieve a pregnancy than those who conceived naturally or with ART (37.3% vs 26.6%, $p = 0.013$) [29]. The findings highlight the clinical relevance of SDF testing in assessment of treatment outcome. Similarly, SDF results were associated with pregnancy rates after varicocele repair in another two studies. The first study evaluated 42 infertile men with high-grade clinical varicocele and impaired semen parameters and 10 normozoospermic fertile men as control. A marked improvement in semen parameters and a decrease in DFI were observed 3–6 months after microsurgical varicocelectomy. DFI in patients who achieved pregnancy after varicocelectomy (20.6% +/− 3.5%) were lower than the results of non-pregnant patients (24.7% +/− 6.5%; $p < 0.01$) and preoperative levels (27.4% +/− 6.3%; $p < 0.01$) and were not significantly different from controls (11.5% +/− 3.9%) [30]. Another study prospectively evaluated 75 infertile men with clinical varicocele and abnormal semen parameters. Couples with positive pregnancy outcome at 1-year follow-up had significantly lower DFI than those who did not (16.4% vs 24.7%; $p = 0.04$) [31]. In contrary, other studies failed to demonstrate an improvement in pregnancy outcomes after varicocele repair despite a significant decrease in SDF [32, 33].

However, the small patient number [32] and methodology of SDF assay [33] were the potential pitfalls.

In summary, a number of studies have provided strong support for a significant decrease in SDF after varicocelectomy in the majority of patients with clinical varicocele and impaired semen parameters. It is important to note that the reduction in SDF as early as 3 months post-varicocelectomy predicts pregnancy outcomes, both natural and assisted, at 1–2 years. Current evidence supports the potential value of SDF testing in clinical practice, though further research is essential in refining the role of SDF assays in preoperative selection of surgical candidates who will benefit from varicocele repair.

Clinical Utility of SDF Testing in Men with Varicocele

While semen analysis remains the cornerstone for assessment of infertile men with varicocele, the potential role of SDF assay in better management decision is emerging. Although major guidelines issued by professional societies still recommend varicocele repair in infertile men with clinical varicocele and abnormal conventional semen parameters [5–7], the incorporation of advanced sperm function tests including SDF was acknowledged in the latest updates [6, 7]. More importantly, the Society for Translational Medicine (STM) has recently published clinical practice guidelines for SDF testing in infertile men in an evidence-based approach. SDF testing is recommended in patients with grade 2/3 varicocele with normal conventional semen parameters and patients with grade 1 varicocele with borderline/abnormal conventional semen parameters [8]. The essence of the recommendations is to provide guidance to the management of highly controversial situations where surgical intervention may not be warranted based on current guidelines. The clinical scenarios proposed in STM guidelines highlight the deficiency of the current recommendations which were mainly based on conventional semen parameters. These are exactly the potential areas where SDF testing may better select varicocele candidates for early surgical interventions.

SDF testing enables the evaluation of sperm chromatin for the first time. The assessment of sperm quality opens an important area for further research and offers opportunity to investigate another facet of male gamete which is not covered by conventional semen parameters [34]. The expanded indications and possible early selection of surgical candidates made possible by SDF assays potentially halt further deterioration of fertility. The progressive impairment in spermatogenesis over time in patients with varicocele has been reported [35]. The benefits of preventing varicocele-associated progressive testicular dysfunction by varicocele repair probably outweigh the minimal risk associated with the advent of microsurgical varicocelectomy. This beneficial effect will be particularly important for couples desiring to have more than one child. In addition, the predictive value of SDF testing on natural pregnancy outcome signified by its strong correlation with time-to-pregnancy in first pregnancy planners provided a solid basis for application of SDF complementary to semen analysis. Men with varicocele should undergo SDF testing together with semen analysis. The results of which will be essential for proper counselling of infertile men and provide another piece of information for management decision. Another important role of SDF test results is the assessment of treatment efficacy after varicocelectomy. This is supported by the correlation between postoperative SDF and natural or assisted reproductive outcomes [29–31]. This is particularly useful for infertile couples of advanced age when postoperative SDF at 3–6 months may predict long-term fertility outcome. Couples who failed to achieve significant SDF improvement after varicocele repair should consider further intervention early.

Conclusion

In light of the emerging evidence over the last few decades, SDF testing should be applied in men with varicocele. SDF is not a perfect test. Its performance depends on appropriate patient selec-

tion and interpretation of results, as all other diagnostic tests. It is more important to recognize the current drawback of using conventional semen parameters as the major selection criteria for varicocelectomy candidates and the potential of SDF testing in rectifying the situation. The development of SDF testing allows the assessment of the most important component of the male gamete for the first time. The limitation of SDF testing should not be considered a hindrance for its utilization as we believe that further exploration via wide clinical application of the SDF testing is the way to refine its clinical value. Rather than continuing with the flawed traditional tests and limit the success in diagnosis and treatment of men with varicocele, it is the time to embrace a promising and innovative male fertility test which may lead to better care of infertile couples.

Review Criteria

An extensive search investigating varicocele and sperm DNA was performed using search engines including ScienceDirect, Ovid, PubMed, and MEDLINE. The study identification was based on the following keywords: "varicocele," "varicocelectomy," "sperm DNA damage," and "sperm DNA fragmentation." The start and end dates for the searches were January 2000 to January 2018, respectively. Only articles published in English were considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included.

Multiple Choice Questions and Answers

1. Sperm DNA fragmentation testing result:
   (a) Correlates closely with conventional semen parameters
   (b) Differentiates fertile and infertile men with varicocele
   (c) Predicts assisted reproductive outcomes particularly intracytoplasmic sperm injection
   (d) **Should be interpreted with conventional semen parameters**
2. Sperm DNA fragmentation is *not* associated with:
   (a) Time-to-pregnancy in first pregnancy planners
   (b) Natural pregnancy rate
   (c) **Fertilization rate of intracytoplasmic sperm injection**
   (d) Miscarriage rate
3. Presence of varicocele is associated with:
   (a) Higher SDF in fertile men
   (b) Higher SDF in infertile men
   (c) Higher SDF in adolescent
   (d) **All of the above**
4. Recent clinical practice guidelines recommend SDF testing in patients with:
   (a) Subclinical varicocele
   (b) Low-grade varicocele with normal semen parameters
   (c) **High-grade varicocele with normal semen parameters**
   (d) High-grade varicocele and azoospermia
5. Potential benefits of clinical utilization of SDF testing does *not* include:
   (a) **Identification of underlying etiology of infertility**
   (b) Better identification of varicocelectomy candidates
   (c) Earlier intervention which may possibly prevent progressive testicular dysfunction
   (d) More accurate prediction of efficacy of varicocelectomy

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41 Cons: Should Sperm DNA Fragmentation Testing Be Used in Men with Varicocele?

Mark Sigman and Danielle Velez

Key Points

- While populations of men with varicoceles have been found to have greater levels of sperm DNA fragmentation than men without varicoceles, the absolute levels of fragmentation are often in the "normal" range.
- Current SDF testing suffers from lack of standardization and an absence of universally accepted thresholds.
- There is insufficient data to conclude preoperative SDF testing can be used to select which patients will benefit from varicocele repair.
- While there is evidence of improvement of sperm DNA integrity after varicocelectomy, there are no robust studies correlating improvement in SDF to live birth rates.
- Currently there is no role for SDF testing in patients with a subclinical varicocele.

Sperm DNA Fragmentation and Varicocele

As intracytoplasmic sperm injection (ICSI) has gained success and popularity, attention has shifted from sperm function, traditionally tested with semen analysis, to the evaluation of sperm DNA integrity. Sperm DNA is highly compacted, relying on underlying protamines and, to a lesser extent, histamines to protect it from damage during transport. While the ova can repair some DNA nicks, extensive damage can overwhelm the cell, resulting in compromised fertilization, embryo development, and pregnancy [1]. Sperm DNA integrity is measured as sperm DNA fragmentation (SDF) or the percentage of cells with chromatin defects (DFI%).

SDF is caused by many factors. If the concentration of reactive oxygen species exceeds that of available antioxidants, developing sperm are exposed to higher levels of oxidative stress. Free radicals can result in single- and double-stranded DNA breaks. This is compounded by nuclear protein defects, causing poor chromatin packaging, exposing greater lengths of DNA to abnormal methylation, nicks, cross-links, and base modifications. These factors can lead to an overwhelming amount of apoptosis, which results in the recruitment of inflammatory cells, further perpetuating the environment of oxidative stress.

Several studies have shown that men with varicocele and male factor infertility have greater

M. Sigman (✉)
Department of Surgery (Urology), Brown University/Lifespan, Providence, RI, USA
e-mail: Mark_Sigman@brown.edu

D. Velez
Department of Urology, Brown Urology/Rhode Island Hospital, Providence, RI, USA

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levels of reactive oxygen species. Although the exact relationship is unclear, there are many hypotheses for how a varicocele may induce greater oxidative stress. The abnormally dilated, tortuous veins of the pampiniform plexus cause an average increase of scrotal temperature of 2.6 °C. This negatively impacts the temperature-sensitive process of spermatogenesis. Higher levels of cytokines have been found in the semen of men with varicocele. Cytokines are pro-inflammatory activators, leading to the generation of reactive oxygen species. Reactive oxygen species cause lipid peroxidation, breaking down the sperm membrane and introducing DNA breaks. Elevated levels of nitric oxide, which are hypothesized to increase testicular arterial flow in response to the venous stasis of a varicocele, provide the ingredients for peroxynitrite and peroxynitrous acid, other potent oxidants [2].

Ultimately, the literature has established that a high level of sperm DNA fragmentation is a negative predictor for natural conception and assisted reproductive outcomes. Evenson et al. [3] measured the SDF of couples who desired pregnancy using the sperm chromatin structure assay (SCSA). Of the couples who achieved pregnancy within the first three months of discontinuing contraception, none had >15% denatured spermatozoa. SDF was significantly greater in couples achieving pregnancy in months four to 12 compared to their cohort who achieved pregnancy in under 3 months, and no couple with denatured spermatozoa >30% achieved pregnancy. As a result, DFI% greater than 30% is often considered the upper threshold of fertility. However, this has not been consistently proven within the literature, thereby establishing the first major drawback to the use of DNA integrity testing: in its current state, there lacks a limit of DFI% at which point couples are deemed infertile.

Sperm DNA fragmentation within the varicocele population has been extensively studied, although with conflicting results. Zini et al. [4] conducted a systematic literature review and identified 16 case-control studies comparing men with varicocele to men without. The authors noted that in six of the studies, men with varicocele and no prior infertility had higher levels of SDF than their fertile counterparts without varicocele. However, five studies found similar levels of SDF in infertile men with and without varicocele, making it difficult to attribute the poor sperm DNA integrity to varicocele alone. Much like traditional semen analyses, the same DFI% does not absolutely distinguish the fertile from the infertile male.

A meta-analysis, performed by Wang et al. [5], compared 240 men with varicocele to 176 men without varicocele and reported that overall, men with varicocele have a higher DNA fragmentation index than men without varicocele, with an average SDF difference of 9.84%. However, there was a wide range of DFI%, with four of the eight included studies having a DFI% of <10% for both the experimental and control groups.

The Wang et al. meta-analysis raises several important questions of SDF threshold for infertility – if half of the infertility group within this meta-analysis already has a low DFI%, what additional value does SDF testing provide over traditional semen analysis prior varicocelectomy? Within this meta-analysis, three different assays were used to measure SDF, making it difficult to draw comparisons and therefore conclusions. Does the threshold for fertility change depending on the assay performed? Is one more accurate than the other? With sperm DNA fragmentation testing within its early stages, there is an opportunity for standardization of testing and reporting of results, which should lead to the establishment of an evidence-based consensus of normal parameters for DFI%.

Varicocelectomy Impact on SDF

While no cause-and-effect relationship has been established between varicocele and male infertility, there is certainly an association, given the higher prevalence of varicocele within the male infertility population. The AUA recommends that varicocele repair should be offered to couples with male factor infertility, if the male partner has a palpable varicocele and an abnormal semen analysis. While the majority of men will experi-

ence an improvement in semen analysis parameters following varicocele repair, pregnancy and live birth are not as consistently achieved after intervention. In addition, some with varicoceles have normal semen parameters but may have impaired sperm function.

This has led to the quest for better screening to determine which men with suspected male factor infertility and clinical varicocele will be best served with varicocelectomy – specifically which men will develop improved semen parameters or sperm function from varicocelectomy. Ideally, there would be an established cutoff of DFI%, as well as a predicted average improvement in SDF post-varicocelectomy. The meta-analysis by Wang et al. [5] reported that varicocelectomy improved sperm DNA integrity by an average of 3.37%. This was significantly less than the 10.7% decrease in SDF reported by the nonrandomized case-control report by Abdelbacki et al. [6], as measured using the SCSA assay, in 60 infertile men with clinical varicocele versus 20 healthy, fertile controls.

While the literature provides evidence of improvement of sperm DNA integrity after varicocelectomy, this has yet to be verified in randomized studies to rule out normal biologic variation in DFI%. Furthermore, the decrease in SDF has yet to show a correlation with improved pregnancy and live birth rates.

Does SDF Testing Have Value in the Varicocele Population?

Several factors go into recommending varicocelectomy for a couple with male factor infertility. Perhaps the most important variable is age of the female partner, as this imposes a shortened timeline for a couples' fertility potential. If natural conception is desired and feasible, a test that would better predict which patient is expected to achieve the most benefit from varicocelectomy would greatly improve patient counseling. Furthermore, if the couple has already committed to assistive reproductive technology (ART), the question shifts to whether pregnancy and live births are increased if varicocelectomy is performed prior to sperm retrieval. In reviewing the literature, two studies attempted to describe which men would most benefit from varicocele repair.

Smit et al. [7] studied the effect of varicocelectomy on sperm DNA fragmentation and semen analysis. Semen analyses and SDF were compared for 49 men with clinical varicocele and ≥ 1 year of infertility before and after varicocelectomy. Average DFI% pre-varicocelectomy was 35.2%. Sixty-three percent of the group were labeled "responders," with at least a > 50% improvement in sperm concentration and had a lower average post-varicocelectomy DFI% than non-responders: 28.6% versus 33%. Although this study did show a small decrease in SDF with varicocelectomy, the only preoperative factor significantly predictive of pregnancy was preoperative sperm concentration, again calling into question the utility of SDF over traditional semen analysis variables.

Abdelbacki et al. [6] also created a logistical regression model using data from their prospective, cohort study to identify pre-varicocelectomy variables of men who stand to benefit the most from varicocelectomy. Preoperative ROS, DFI%, and infertility duration were the most different variables between the infertile varicocele population and healthy controls. The model had an overall success rate of 83.6%, identifying 90% of patients whose semen analysis improved with varicocelectomy, and 66.7% of those who did not. The authors found that every one-point increase in DFI% correlated to a decreased chance of improvement in fertility by a factor of 1.4. While this is the most promising study to date on clarifying the role for SDF testing prior to varicocelectomy, it has yet to be reproduced in larger studies and, again, does not measure the ultimate outcome of pregnancy and live birth. In addition, the conclusion argues against using high DFI as an indication for varicocele repair. It suggests just the opposite – that varicocele repair may be contraindicated in those with elevated DFI. There are no studies demonstrating improved semen parameters or pregnancy or live birth rates when DFI is used to select which varicocele patients should undergo varicocele repair.

Role of SDF in Subclinical Varicocele

The AUA recommends varicocele repair only in men with abnormal semen parameters and a clinical palpable varicocele. However, there is data within the literature in support of varicocelectomy for subclinical, or non-palpable, varicocele. Yamamoto et al. randomized 85 infertile men with subclinical varicocele to high ligation or observation and, after 1 year, found no significant difference in pregnancy rates, but significant improvements in total motile counts and sperm density within the intervention group [8].

Garcia-Peiro et al. [9] compared the percentage of DNA fragmentation between different grades of varicocele within a cohort of infertile males. The authors found that clinical and subclinical varicocele patients had higher DFI% than the fertile controls without varicocele. They also noted within the subclinical varicocele group that underwent varicocele repair, SDF levels did not significantly improve. This is in line with other studies, which have shown a positive correlation between grade of varicocele and DFI%. A 2017 guideline statement from the Society for Translational Medicine recommended against SDF testing in patients with infertility and non-palpable varicocele [10].

Weaknesses in Current Literature

While there is certainly evidence of an inverse relationship between DNA fragmentation and fertility, the literature is still lacking in many areas to support routine DNA integrity testing in the evaluation of the infertile male and is almost nonexistent to support utilizing SDF testing to select the patients that would most benefit from varicocele repair.

First, laboratory tests require cutoff values to define abnormal from normal. While some studies suggest that SDF of >30% is unlikely to result in pregnancy during natural conception, there is no agreed-upon value in patients undergoing ART [10], and indeed, there have been reports of pregnancies, both naturally and via ART, with documented SDF of >30%. This calls into question the accuracy and comparability of the assays, as well as the true SDF threshold for fertility.

The literature is further complicated by the challenge of finding studies with an adequate study design to properly answer the question of the value of SDF testing in the varicocele population. While many studies report on the treatment of men with palpable varicoceles in couples unable to conceive a child for ≥12 months, there is often no control group or the control group may include men who have had children within 12 months of unprotected intercourse, those with or without a clinical or subclinical varicocele, or sperm donors. The control group that is needed are those infertile men with palpable varicoceles – the same as the treatment group. The analysis needs to compare those with and without preoperative elevations in SDF and examine the defined outcomes of pregnancy and live birth with and without varicocele repair.

Ultimately, easily measured endpoints are vital to conducting meaningful research. For studies on infertility the most appropriate outcomes are pregnancy and live birth. Unfortunately, most data utilized changes in semen parameters which are not equivalent biologic endpoints to actual pregnancy. In addition, these biologic endpoints are heavily influenced by maternal factors, socioeconomics, lifestyle, etc.

Finally, the results of any diagnostic test should provide the clinician with a treatment strategy. The greatest weakness to SDF testing is the shallow arsenal of treatment options. It is unclear why some patients' post-varicocele repair have greater improvement in their DFI% than others. After varicocele repair, there are no evidence-based treatment options to further reduce oxidative stress within the testicular microenvironment to facilitate pregnancy. Furthermore, as of now, the literature cannot explain how patients with a "normal" DFI% post-varicocele still require ART for conception or even still fail ART. Just as the link between varicocele and infertility is likely multifactorial, there are other infertility variables that must be identified and considered in the treatment of male factor infertility. Until further, well-designed studies are published, there is insuf-

ficient data to support a clinical role for SDF testing in the varicocele population. We are still dependent on the guidelines/best practice statements of all of the major infertility organizations which recommend using the history, physical exam (for palpable varicocele detection), and semen parameters to choose those patients best suited for varicocele repair.

Conclusion

Current data does not support a routine role for SDF testing in the varicocele population.

Review Criteria

An extensive search of studies examining the relationship between varicocele, oxidative stress, sperm DNA integrity, and varicocelectomy was performed using search engines such as ScienceDirect, PubMed, and MEDLINE. The start and end dates for these searches were January 2011 and May 2018, respectively. The overall strategy for study identification was based on the following keywords: "varicocele," "oxidative stress," "reactive oxygen species," "varicocelectomy," "male infertility," and "pregnancy rate." Only articles published in English were considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included.

Multiple Choice Questions and Answers

1. All of the following are theories on the relationship between varicocele and sperm DNA fragmentation, except:
   (a) Oxidative stress is thought to be the leading cause of impaired sperm DNA integrity due to higher radical oxygen species, which leads to single- and double-stranded DNA breaks
   (b) The venous stasis of varicoceles creates a warmer environment, on average 2.6 °C, negatively impacting spermatogenesis
   (c) **Increased concentrations of nitric oxide, which are thought to occur in varicoceles in response to the venous stasis, act to combat oxidative stress, protecting spermatogenesis**
   (d) The more single- and double-stranded DNA breaks present, the harder it is for DNA to appropriately compact around protamines, exposing greater lengths of DNA to oxidative damage.
2. SDF testing of varicocele patients:
   (a) Predicts which patients will have orchialgia relieved by varicocelectomy
   (b) Identifies those patients that will have varicocele recurrence after varicocelectomy
   (c) Always shows levels of SDF > 25%
   (d) **Has yet to demonstrate utility in selecting patients for varicocelectomy**
   (e) Is only useful if performed with the COMET assay
3. What is the average improvement in sperm DNA fragmentation after varicocelectomy?
   (a) 5%
   (b) 10%
   (c) 20%
   (d) 25%
   (e) **There is no industry agreed-upon improvement in SDF after varicocelectomy**
4. What is the most often generally accepted threshold of sperm DNA fragmentation, after which pregnancy is highly unlikely?
   (a) >10%
   (b) **>30%**
   (c) >40%
   (d) >50%
   (e) >75%
5. There is a need for tests of fertility beyond the semen analysis because:
   (a) **The semen parameters of fertile and infertile populations greatly overlap.**
   (b) The semen parameters of patients with varicoceles are rarely impaired.
   (c) The American Urology Association (AUA) and the American Society of

Reproductive Medicine (ASRM) do not recommend semen analyses in the evaluation of the male with a varicocele.

(d) Pregnancy but not birth rates correlate with sperm counts.

(e) Most fertile men have semen parameters below the reference ranges utilized by the World Health Organization (WHO).

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42 Pros: Should Sperm DNA Fragmentation Testing Be Used in Men with Varicocele?

Nannan Thirumavalavan, Joseph Scott Gabrielsen, and Alexander W. Pastuszak

Key Points

- Varicocele repair will allow some couples to achieve spontaneous pregnancy and avoid ART and allow some couples to use less expensive and invasive forms of ART.
- Varicoceles are progressive lesions that result in testicular dysfunction and infertility in some men.
- Varicocele repair improves sperm quality and quantity, resulting in improved natural pregnancy outcomes, as well as outcomes after IUI and IVF/ICSI.
- Varicocele repair is more cost-effective than proceeding directly to ART.
- Men with infertility should be evaluated by a male fertility specialist, and when a varicocele is identified on evaluation, couples should be counseled regarding the risks and benefits of varicocele repair prior to proceeding to assisted reproductive technology.

Introduction

Varicoceles are the most common cause of male infertility, and varicocele repair (VR) was one of the earliest treatments for male infertility, first being reported in the 1950s [1]. Since then, the treatment of infertility has been revolutionized through the advent of assisted reproductive technologies (ART), including intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI). With the ability to create an embryo using a single sperm, the role of VR in patients utilizing ART is heavily debated. However, current evidence, as reviewed in this chapter, reveals that varicocele repair prior to ART can be beneficial. Couples with men undergoing varicocele repair may be able to avoid ART completely and become pregnant naturally or utilize less invasive and less expensive methods of ART to achieve a pregnancy than they would have been able to in the absence of repair. For couples who require ART, evidence supports that varicocele repair improves outcomes. Therefore, clinical varicocele should be repaired prior to proceeding with ART.

N. Thirumavalavan (✉)
Urology Institute, University Hospital / Case Western Reserve University, Cleveland, OH, USA
e-mail: nannan@bu.edu

J. S. Gabrielsen
Division of Male Reproductive Medicine and Surgery, Scott Department of Urology, Baylor College of Medicine, Houston, TX, USA

A. W. Pastuszak
Department of Surgery, Division of Urology, University of Utah School of Medicine, Salt Lake City, UT, USA

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Table 42.1 Indications for varicocele repair prior to ART

Male with a varicocele in a couple with infertility *and* one (1) of the following:
Decreased sperm density or total sperm count
Decreased sperm motility
Abnormal sperm morphology
High DNA fragmentation
High reactive oxygen species content

Table 42.2 Improvements after varicocele repair

Decreased need for ART
Increased success rates using ART
Increases in testosterone levels
Improved sperm quality
Increased natural pregnancy rates
Increased sperm retrieval rates in azoospermic men

Varicocele Repair May Eliminate or Decrease the Need for ART

If a surgical intervention allows patients to achieve pregnancy using a less invasive or more cost-effective approach, this is a meaningful improvement. Patients commonly ask what level of improvement can be expected from VR. Though the degree of improvement can vary between patients, VR can improve sperm counts, motility, and quality enough to facilitate the use of less invasive and less expensive forms of ART [2, 3] (Tables 42.1 and 42.2). For example, a couple initially relegated to IVF as a function of total motile sperm count may have enough of an improvement in total motile count after VR that permits the use of IUI or even natural conception. Cayan et al. reported a series of 540 patients. Of those initially limited to IVF or ICSI, 31% became either IUI or natural pregnancy candidates after VR [4]. Of the patients initially limited to IUI, 42% had improvements in sperm counts that could facilitate a natural pregnancy. Similarly, Samplaski et al. reported on a series of 373 men undergoing varicocele repair and observed that of the men with semen parameters placing them in the IVF category, VR resulted in 21.6% being "upgraded" to the IUI category and 31.7% being "upgraded" to the natural pregnancy category. Of men with semen parameters making them IUI candidates, 57.6% "upgraded" to the natural pregnancy category [3]. Though these numbers are promising and enable practitioners to tell patients what ART they are candidates for, the true outcomes lie in whether pregnancies were actually achieved using less intense ARTs.

To address this limitation, studies have investigated pregnancy rates after varicocele repair. Daitch et al. reported a series of 58 patients undergoing IUI and observed that pregnancy and live birth rates per cycle were higher in patients who underwent varicocele prior to IUI [5]. Similarly, Esteves et al. compared success rates in couples using ICSI between those with male partners undergoing VR and controls [6]. The authors observed that clinical pregnancy and live birth rates were higher in the VR group, with an odds ratio (OR) of 1.82 (95% CI 1.06–3.15) for pregnancy and 1.87 (95% CI 1.08–3.25) for live birth after VR. Also, the incidence of miscarriage was approximately halved in couples in whom the male partner underwent VR. Zini et al. followed 610 couples in whom the male had a varicocele and compared patients who underwent VR to those who did not [7]. Natural and ART pregnancy rates were not statistically different between the two groups, but patients who underwent VR utilized ART in 38% of cases, whereas 54% of patients who did not undergo repair required ART ($p < 0.05$). A significant reduction in the need for ART after VR could translate into lower cost and shorter time to pregnancy for many couples. Avoiding IVF significantly decreases the burden on the female partner given the risks of IVF, including multiple pregnancy, hyperstimulation, and ovarian torsion [8]. Albeit small, there is also a slightly increased risk of birth defects in children conceived using IVF and complications associated with multiple gestations, which are avoided if couples do not utilize IVF/ICSI [9–12].

Varicocele Repair Improves Sperm Quality

Physicians and patients may have difficulty accepting that varicocele repair may improve outcomes when patients already have sperm in the ejaculate. Since patients have sperm in the ejaculate, the misconception is that the easiest and most direct path to successful pregnancy is IUI or IVF after unsuccessfully trying to achieve natural

pregnancy. However, VR can result in improved sperm concentration, motility, and morphology, all of which can improve ART outcomes [13, 14]. Along the same lines, a common question is the mechanism by which VR improves sperm quality. The mechanism of testicular damage in varicocele may be from a combination of testicular temperature elevation, pressure effect, oxygen deprivation, and retrograde reflux of gonadal toxins from incompetent veins (Fig. 42.1) [15]. These abnormalities lead to Leydig cell and Sertoli cell dysfunction, as evidenced by decreases in antimullerian hormone (AMH), inhibin-B, transferrin, androgen-binding protein (ABP), and testosterone levels [16–19]. A large European study demonstrated that unselected (not infertile) men with asymptomatic varicoceles found on routine physical exam had decreased sperm counts, motility, and morphology [16]. Infertile patients with varicocele have morphologically abnormal sperm, with lower percentages of normal forms and more elongated, tapered sperm heads and amorphous cells [13, 20]. In addition, patients with varicocele may have genetic or biochemical mechanisms that contribute to the pathogenesis of varicocele-associated subfertility [21]. These mechanisms include mutations in genes including HIF-1alpha, DNA topoisomerases, and polymerases, as well as genes that are responsible for hormone metabolism and make patients more susceptible to oxidative stress, hormonal imbalances, exogenous toxins, heat stress, hypoperfusion, and hypoxia, which then leads to infertility.

VR partially reverses some of the above dysfunctions and defects. Baccetti et al. found that after VR, men have an improvement in sperm morphology as assessed by electron microscopy and improvement in sperm FISH findings [22]. Other tests of sperm quality, such as DNA fragmentation and reactive oxygen species (ROS),

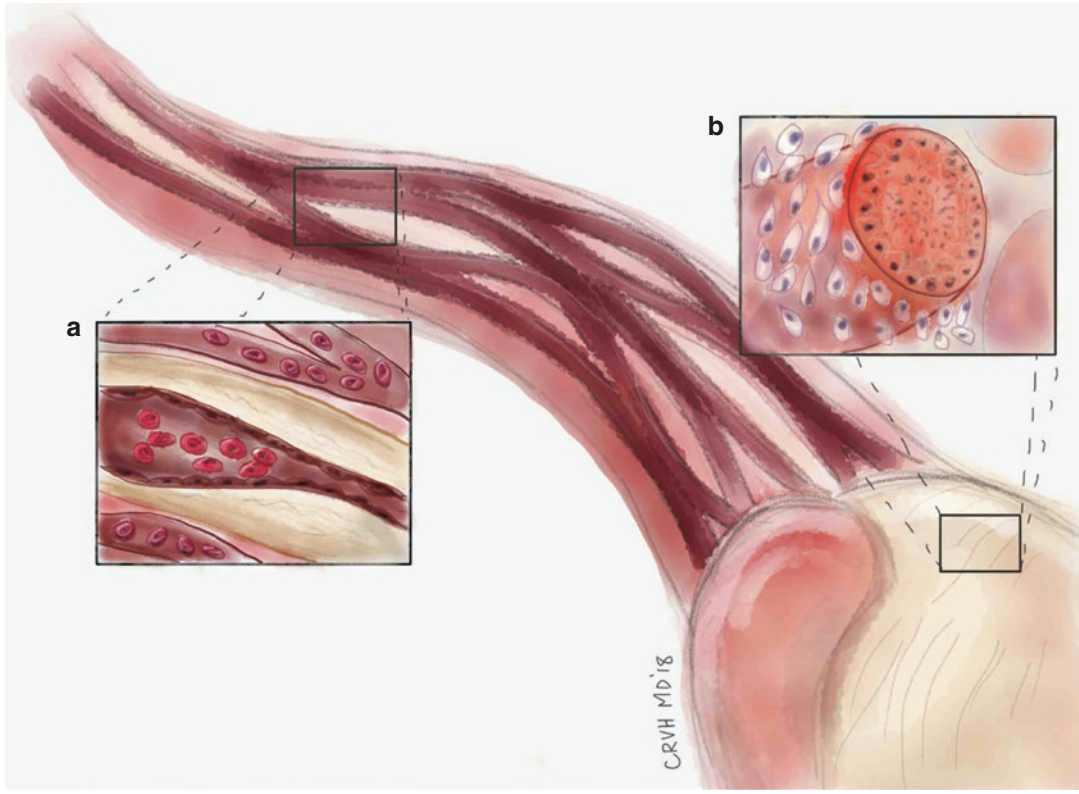

Fig. 42.1 Mechanisms of varicocele effects on the testicle. (**a**) Elevated temperature, venous stasis, decreased arterial flow, hypoperfusion, and hypoxia. (**b**) Increased oxidative stress, reactive oxygen species, hormonal imbalances (increased LH and FSH, decreased testosterone), increased accumulation, and decreased clearance of waste products. (Illustration Acknowledgement: Christopher R.V. Hoover, MD)

have also shown improvements after VR [23–27]. These are especially important for patients requiring IVF and ICSI. A pilot study showed that sperm mitochondrial DNA quality improves after VR as well [28]. Because DNA quality is not routinely assessed by standard semen analysis, infertile men with normal semen parameters and a varicocele can likely benefit from repair. Ghanaie et al. investigated couples with recurrent miscarriages in which the male partner had a varicocele, but normal semen parameters [29]. Compared to an observation group, the authors found that patients undergoing VR had significantly fewer first trimester miscarriages and higher pregnancy rates. As such, aside from quantity alone, VR improves sperm quality, which leads to better outcomes.

Outcomes in Non-azoospermic Patients

Infertile men represent a heterogenous population, with baseline semen parameters varying widely. We will review the evidence for VR in each population and demonstrate that VR benefits multiple populations of infertile men.

Opponents of VR will contend that if there is sperm present, the easiest and quickest course of action would be to proceed to ART. Unfortunately, many patients with sperm in their ejaculate proceed along this pathway without knowledge that they even have a varicocele. However, studies have revealed that outcomes after ART are better after VR. In one study looking at 58 couples, pregnancy and live birth rates per IUI cycle were significantly higher in couples in which the male partner underwent VR (11.8% vs. 6.3%, $p = 0.04$, and 11.8% vs. 1.6%, $p = 0.007$, respectively) [5]. When looking at patients who proceed to IVF, multiple studies have found that pregnancy rates are improved with VR [6, 30–32]. In a comparison of 169 oligozoospermic men after VR to 79 men with untreated varicoceles, Pasqualotto et al. reported an increased fertilization rate in the VR group, but no difference in pregnancy rates [33]. However, they also reported a decrease in time to pregnancy in the VR group by almost 3 years. The significant improvement in multiple outcomes supports the conclusion that men with sperm in the ejaculate in couples with fertility difficulties should undergo VR prior to attempting ART.

Outcomes in the Infertile Male with Non-obstructive Azoospermia (NOA)

In the non-obstructive azoospermic (NOA) male, some physicians argue that proceeding straight to a microsurgical testicular tissue extraction (micro-TESE) is appropriate. However, patients with NOA and a varicocele should consider VR. The best outcome in this situation is when VR can result in sperm in the ejaculate after repair and eliminate the need for micro-TESE. In one series of 83 patients with NOA and a left-sided varicocele who underwent varicocelectomy, 20 (24%) had sperm return to the ejaculate within 1 year after surgery [34]. Testicular histology of hypospermatogenesis or maturation arrest portends a higher likelihood of return of sperm into the ejaculate when compared to Sertoli cell-only histology. Another series reported a 22% (7/31) likelihood of return of sperm into the ejaculate after microsurgical VR [35]. Similarly, Weedin et al. performed a meta-analysis of 233 patients with NOA who underwent VR and found that 91 patients (39.1%) had sperm return to the ejaculate. The authors also found that testicular histology of hypospermatogenesis or maturation arrest was associated with a higher likelihood of return of sperm into the ejaculate. Since up to 39% of patients could avoid a micro-TESE, varicocele repair in NOA patients is certainly warranted and beneficial for patients.

For the remainder of patients who do not have sperm return to the ejaculate after varicocele repair, a significant benefit still exists. When those patients proceed to micro-TESE, sperm retrieval rates are improved. In Shiraishi et al.'s study of 53 NOA patients undergoing micro-TESE, sperm retrieval was successful in 19, or 36% of patients [34]. Kirby et al. performed a meta-analysis of two separate clinical trials comparing sperm retrieval rate after VR compared with no varicocele intervention and found that

sperm retrieval rate was improved among those having undergone VR (OR = 2.509, $P = 0.0001$) [36, 37]. In another meta-analysis comparing sperm retrieval rates in 159 NOA patients after VR to 241 patients undergoing observation for their varicocele, Esteves et al. observed a higher sperm retrieval rate after VR (OR 2.65; 95% CI, 1.69–4.14; $P < 0.0001$) [38]. In summary, patients with NOA and a varicocele will likely benefit from VR, either by eliminating the need for micro-TESE or with improved outcomes from micro-TESE.

The most significant outcomes after any treatment or intervention for fertility are pregnancy and live birth rates. However, these outcomes are often the most difficult to track due to loss to follow-up after male fertility treatments. It is important to differentiate between natural pregnancy rates (not requiring ART) and pregnancy rates after IUI and IVF. In their meta-analysis, Weedin et al. reported a 6% natural pregnancy rate (14/233) for all NOA patients who underwent VR [15]. However, an additional 10 pregnancies were achieved with the use of IVF in this cohort. Also looking at NOA men, Shiraishi et al. reported a 6% (5/83) pregnancy rate from either "timing or male-assisted insemination pregnancy" [34]. Kirby et al.'s meta-analysis of two studies looking at pregnancy rates after VR compared to no intervention in azoospermic men found an odds ratio of 2.336 (1.022, 5.342, $p = 0.044$) favoring VR [30, 36, 37]. In summary, male partners with NOA and a varicocele should undergo VR, as this may result in natural pregnancy, an improved likelihood of success during micro-TESE, and most importantly an improved live birth rate when compared with NOA men with varicocele who do not undergo VR.

Cost-Effectiveness of Varicocele Repair

The cost of any intervention for infertility is a significant consideration for most patients, especially because many treatments may not be covered by insurance. With only six states (CA, CT, MA, NJ, NY, and OH) having state mandates for insurance coverage of male infertility, many expenses are borne out of pocket for couples [39]. Fortunately, VR is significantly cheaper than ART. Using data from 1994, Schlegel et al. reported that median fees, including pretreatment fees, the surgical fee, anesthetic fee, ambulatory surgery fee, as well as time lost from work and delivery costs, totaled approximately $26,268 per delivery [40]. Comparatively, costs for ICSI without VR totaled $62,263 per delivery. Cayan et al. compared cost-effectiveness of four treatment arms – observation, VR followed by up to three cycles of IVF if the couple did not conceive naturally in the year after surgery, up to three cycles of gonadotropin superovulation and (IUI) followed by up to three cycles of IVF if IUI failed, and up to three cycles of immediate IVF [41]. The authors found that the VR arm cost $52,000 per additional live birth, while proceeding directly to IUI/IVF cost $561,000 per additional live birth. Other analyses have found similar results [42, 43]. From the patient perspective, VR is more likely to be covered by insurance than ART is. Though cost should not be the primary consideration when counseling patients regarding treatment options, the cost-effectiveness combined with better outcomes make a strong argument for patients to undergo VR prior to ART.

Varicocele Repair May Stop Progressive Testicular Decline

In most clinical scenarios, patients presenting with infertility and varicocele are focused on addressing the most imminent problem – having a child. However, physicians taking care of these couples should also keep in mind the couple's future fertility potential, should they desire another child. Varicoceles have long been considered a progressive lesion, as evidenced by their increased prevalence in patients with secondary infertility [44, 45]. Patients forgoing VR and proceeding to ART may have a progressive decline in testicular function, semen parameters, and importantly fertility potential. Patients should be counseled about the possible worsening of their fertility potential over time, especially if they desire more than one

child. In addition, once ART has been used for the first child, they will likely use ART again for future children. Varicocele repair is performed in adolescents to stop the progressive decline of testicular function [46]. Infertile men may also have concomitant hypogonadism. The varicocele repair not only improves their fertility potential but also may improve testosterone levels [47–50]. In addition, Najari et al. reported a case series of 34 patients undergoing VR [51]. Using validated questionnaires, the authors observed that 44% of men had improvements in gonadal function and 53% had improvements in ejaculatory function. These additional benefits should be given consideration and add to the growing list of reasons to strongly consider VR prior to ART.

Opposition to Varicocele Repair

Most urologists and male infertility specialists agree that VR is warranted prior to proceeding to ART. However, arguments against this approach have been made. One argument emphasizes a potentially longer wait time to achieving pregnancy, particularly in women of advanced maternal age. However, studies support that time to pregnancy is similar, if not improved, in couples in whom the male partner undergoes VR [33, 52]. Even if a longer time to pregnancy did result from VR, many patients may prefer to increase their chances of initiating a pregnancy naturally given the cost and intensity of ART. Some may argue that avoiding VR avoids a surgery and an anesthetic for the male partner. However, a minor outpatient surgery with minimal morbidity and mortality is preferable when it could obviate the need for ART and its associated risks for the female partner. Thus, VR is a better option for a couple as a whole. More significantly, VR is not performed as commonly as it should be given a persistent dearth in male partner evaluation. Most infertile couples are first evaluated by reproductive endocrinologists (REIs), and further male workup is not always initiated by the initial physician seeing the couple. Further, many couples proceed directly to ART when adequate number of sperm are present in the ejaculate. In addition, urologists and male infertility specialists may be reticent to perform VR on patients that have been prepared and counseled for IVF, for fear that this may discourage further referrals. Though the unfortunate reality, this trend should be reversed for the benefit of the patients, and more patients should be evaluated and given the option for VR prior to ART.

Conclusions

Varicocele repair provides a significant benefit for patients prior to ART and can completely avoid the need for ART in some patients and improve outcomes for those patients who require ART. VR may impede the progressive decline of testicular function associated with varicocele and is more cost-effective than proceeding directly to ART. Men with a varicocele and male factor infertility should be given the option to undergo VR prior to any form of ART. The couple should also be presented with the risks and benefits for all their fertility options and allowed to make an informed decision regarding the best way to achieve their fertility goals.

Review Criteria

An extensive search of the studies relevant to varicocele and its role prior to assisted reproductive technologies was performed using PubMed. The literature review was performed in July of 2018. Only data from published articles in English were considered and included.

Multiple Choice Questions and Answers

1. Varicocele repair has been shown to improve the following except:
   (a) Total motile sperm count
   (b) Sperm morphology
   (c) Sperm DNA fragmentation
   (d) Testosterone
   (e) **Urinary function**

2. Which of the following is *not* a benefit of varicocele repair in the azoospermia patient?
   (a) Chance of obtaining ejaculated sperm
   (b) Improved sperm retrieval rates from micro-TESE
   (c) Chance to eliminate need for micro-TESE
   (d) **Relieves obstruction**
3. In previous studies, approximately what percentage of NOA men will have sperm in the ejaculate after varicocele repair?
   (a) 0%
   (b) **0–50%**
   (c) 50%
   (d) 50–100%
   (e) 100%
4. Which the following are other emerging possible indications for varicocele repair?
   (a) Stop progressive testicular decline
   (b) Improve gonadal function, including testosterone
   (c) Improving ejaculatory function
   (d) None of the above
   (e) **All of the above**
5. Which of the following do *not* contribute to cost-effectiveness of varicocele repair prior to assisted reproductive technologies?
   (a) Increased chance of natural pregnancy
   (b) Increased success rates with IUI
   (c) Increased success rates with IVF/ICSI
   (d) Significantly less cost than ART
   (e) **Eliminating need for a female evaluation and workup**

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Con: Should Varicocele Be Repaired Before ART?

43

Samuel J. Ohlander, Rodrigo L. Pagani, and Craig S. Niederberger

Key Points

- Not all men with a clinical varicocele suffer from subfertility.
- There is no diagnostic measure in current clinical practice that may predict which individuals will have a negative sequela or which will see improved fertility potential following repair.
- The heterogeneity of study populations and methodological parameters prevents a clinical consensus on the management of varicoceles in the infertile couple.
- Each couple must be approached uniquely, taking into consideration both male and female factors.

Introduction

Infertility is estimated to affect one in six couples, with a contributing male factor in 50% of cases [1, 2]. Varicoceles are present in 15–20% of post-pubertal men with a 2–3 times increased likelihood of diagnosis in those presenting for infertility evaluation and a nearly 4 times increase in those presenting for secondary infertility [3–5].

Since initially observed by Tulloch, there has been numerous studies hypothesizing the pathophysiology and outlining the negative effect of varicoceles on semen parameters and male fertility, as well as the outcomes of repair; however, not all men with varicoceles suffer from subfertility [6–10]. There is no diagnostic measure in current clinical practice that may predict which individuals will have a negative sequela or which will see improved fertility potential following repair. Despite decades of published literature suggesting improvement in semen parameters and infertility, the heterogeneous study populations and methodological parameters result in a lack of consensus on the management of varicoceles in the infertile couple and, essentially, a clinical dichotomy among male and female reproductive physicians [7, 8, 11–16].

In current clinical practice, the development of assisted reproductive techniques (ART) have been utilized to obviate various factors prohibiting fertilization and pregnancy. Female factors such as tubal obstruction may be bypassed. Intrauterine insemination with ovarian stimulation may combat irregular sperm delivery, diminished sperm counts, or cervical pathology. Additionally, in the setting of azoospermia due to spermatogenic dysfunction or severe

S. J. Ohlander (✉) · R. L. Pagani
C. S. Niederberger
Department of Urology, University of Illinois at Chicago Hospital and Health Sciences System, Chicago, IL, USA

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oligozoospermia, ART via in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) offers couples the opportunity to conceive when they previously had not been able by coupling with surgical sperm extraction.

It is critical that each couple's individual circumstances be considered in clinical management decisions. In isolated female factor infertility, varicocele repair is not indicated. The American Urological Association and American Society of Reproductive Medicine recommend that correction of a varicocele "should be considered when most or all of the following are met: (1) the varicocele is palpable on physical examination of the scrotum; (2) the couple has known infertility; (3) the female partner has normal fertility or a potentially treatable cause of fertility, and time to conception is not a concern; and (4) the male partner has abnormal semen parameters" [17].

The clinical debate that surrounds the repair of a clinically palpable varicocele prior to assisted reproduction focuses on two patient cohorts: the non-azoospermic male and the azoospermic male. We noted the uncertainty of which varicoceles may be clinically relevant and which patients may benefit from repair. The basis of this discussion is when should a varicocele be repaired before assisted reproduction? Should ART bypass repair of a clinical varicocele in the management algorithm?

Pathophysiology

Numerous theories on the pathophysiology and testicular dysfunction secondary to varicoceles exist, with the predominate thought being that there is a thermotoxic effect [18]. The male anatomy is such that the gonads exist externally, allowing the normal scrotal temperatures to maintain 1–2 °C lower than normal core temperatures, depending on countercurrent heat exchange from the pampiniform plexus to cool arterial blood entering the testicle. Men with varicoceles have been found to have higher intratesticular temperatures [18]. Ligation of the varicocele is believed to prohibit reflux and venous pooling, eliminating the detrimental thermotoxicity. Surgical treatment improves semen parameters in 60–80% of cases However, despite improvement in semen parameters, Richardson et al. found average pregnancy rate of just under 40%, which is similar to reported fecundity outcomes, further adding to the debate on repair [19].

The Non-azoospermic Male

In clinical practice, total motile sperm counts are often used to stratify the type of assisted reproduction that may be recommended. Men with TMS <five million are often recommended IVF and >20 million are recommended natural conception [20, 21]. Recent reports by Samplaski et al. used modified thresholds of 5–9 million for IUI and >nine million for natural conception (threshold created using lower limit of WHO 2010 reference ranges) to highlight "downgrading" of ART following varicocelectomy [22]. Current belief is that operative management prior to assisted reproduction may improve counts, and possibly sperm quality, to shift couples to a less invasive form of assisted reproduction and improve success rates. In theory, such options would offer a clear management algorithm. However, the work by Samplaski et al. did not provide any data on pregnancy, time to pregnancy, or live birth rate. Additionally, nearly a quarter of the study population had a decline in semen parameters that moved them to a more invasive form of ART [22]. In reproductive care, management is unique in that it relies upon management of the couple and a very specific outcome: delivery of a healthy child. For better or worse, the clinical timeline often plays an integral part in the reproductive management of a couple, and, therefore, broad application of clinical management studies ought to be applied with such considerations in mind.

Intrauterine Insemination

Kohn et al. recently performed a review and meta-analysis examining assisted reproduction results both before and after varicocelectomy [23]. They aptly discuss the paucity of well-designed

studies evaluating the topic, the limitations on female age reporting and induction protocols in the published literature, and the wide variation in reported per couple pregnancy rates after IUI (7.7–50%). They limited their discussion to primarily three studies, of which we will discuss below, resulting in insufficient evidence for clinical advantage of varicocelectomy before IUI.

Marmar et al. retrospectively investigated the effectiveness of IUI without ovarian stimulation after varicocelectomy in a cohort of 71 infertile men with radiographically confirmed varicoceles [24]. Though there was not direct comparison in their study (cohort categorized by sperm penetration assays), evaluation of their data found 52 men were treated surgically and couples underwent 145 cycles of IUI, compared with 14 men that went untreated prior to 30 cycles of IUI. The data demonstrated pregnancy rates of 2.8% and 6.7%, respectively. While mean female age was noted, there was no data or mention as to whether female age was similar in the couples who were treated versus untreated.

Nearly a decade later, Daitch et al. examined men with clinical varicoceles and abnormal semen parameters in likely the most recognized study on varicoceles and IUI [7]. Thirty-four men underwent repair and 24 were untreated. No improvements in semen parameters were observed, though there were improvements in pregnancy and higher birth rates. This study has been the focus of the argument for repair prior to IUI, but again, these were small numbers, and female age was not reported, offering a potential confounding factor that greatly limits the study utility.

Finally, Boman et al. retrospectively reviewed 118 couples of which the male had a clinically palpable varicocele and abnormal motility, including 10 couples that underwent IUI after varicocelectomy and 10 couples went untreated prior to IUI. The authors found statistical improvement in sperm motility and TMSC after repair, but noted a significant decrease in sperm concentration. While there were five pregnancies in the treatment group compared to one in the untreated group, the small study size did not result in statistical significance.

The current published data does not provide clear support for varicocele treatment prior to IUI. Couples ought to be educated on the potential improvement in outcomes, but broad applicability of varicocelectomy prior to IUI cannot be considered standard of care. We must consider whether statistically significant findings should be considered clinically significant. Large, randomized studies with well-defined cohorts are needed, but in the current landscape of reproductive care, such undertaking seems unlikely.

In Vitro Fertilization

In vitro fertilization, particularly after the advent of ICSI, allowed men with very few sperm the ability to conceive. As a previous meta-analysis concluded that varicocelectomy improves pregnancy rates and live birth rates in natural cycles, there has been an increased number of publications advocating that varicocele repair before assisted reproduction improves the outcome of these procedures [8].

The first study that brought this question was published in 1989 [25]. Ashkenazi et al. distributed 22 men with varicocele and altered seminal parameters in two different groups. The first group included men with varicocele with a combined female factor, and the second group included men with varicocele with no female factor associated. The first group was submitted to varicocele repair after a failed IVF attempt and subsequently had a second IVF procedure. The second group had an operation at the moment of their diagnosis and was followed for a period of 18 months. They have observed a success rate of 20% in group one and 30% in group two after varicocelectomy [25]. Apart from the fact that this was a very small study (only 12 patients were enrolled in group one), there was not any data on female age or the varicocele grade. Furthermore, enrollment was based on ultrasound diagnosis of the varicocele, thus including men with subclinical varicoceles for analysis and, possibly, confounding the outcomes.

In a retrospective review of 242 couples submitted to IVF/ICSI, Esteves et al. compared the

outcome of 80 patients who underwent microsurgical varicocelectomy prior to ART to 162 patients who proceeded to IVF/ICSI without varicocele repair [12]. They reported that the treated group had a clinical pregnancy rate of 60% and live birth rate of 46.2%, as opposed to 45% and 31.4%, respectively, in the non-treated group. The authors found an odds ratio of live birth after varicocele repair of 1.87 [95% CI, 1.08–3.25]. Although the distribution of varicocele grades, male age, and female age were similar in both groups, there was a slight, but not significant, increase in female factor in the second group. It is also worth noting that even though they found 476 couples in whom the male partner had a history of varicocele, they ended up including 242 men, without further explaining why the remaining patients did not meet the inclusion criteria.

In the largest retrospective study conducted to date evaluating the role of varicocele repair before IVF/ICSI, Gokce et al. repaired the varicocele in 168 patients before assisted reproduction (group A), whereas 138 patients with varicocele went to IVF/ICSI directly (group B) [16]. Varicocele grade distribution, mean male age, mean female age, and female factor infertility were similar across the groups. Patients in the treated group had a significantly higher sperm count, sperm motility, and sperm morphology than the untreated group before the IVF/ICSI procedure. They observed that patients of group A had a higher pregnancy rate (62.5% vs. 47.1%, $p = 0.001$) and higher live birth rate (47.6% vs. 29%, $p = 0.0002$) than patients of group B. In the logistic regression analysis, varicocelectomy was found to increase pregnancy and live birth rate (OR 2.02, 95% CI 1.25–3.87; $p = 0.032$ and OR 2.12, 95% CI 1.26–3.97; $P = 0.026$).

Contradictory to the previous two studies, Pasqualotto et al. failed to demonstrate the advantage of performing varicocele repair prior to IVF [15]. All subjects had grade III varicoceles and underwent a subinguinal varicocelectomy with magnification. Again, two cohorts were formed, the first one comprised of 79 untreated patients and the second one contained 169 men who have undergone varicocelectomy prior to IVF/ICSI. The cohorts were similar in regard to male and female age, but the treated group had a significantly higher time to conceive, suggesting that other factors may have contributed to infertility in those couples. Once again, nothing was mentioned about possible female factors involved in those couples. They reported a higher fertilization rate in the untreated group (73.2% vs. 64.9%, $p = 0.0377$); however, the pregnancy rates, implantation rates, and miscarriage rates were similar in both groups.

Recently, two meta-analyses have been published assessing the outcomes of assisted reproduction technology (ART) in men with treated and untreated varicoceles. Esteves et al. preformed a systematic review and meta-analysis on the outcomes of ICSI in non-azoospermic men with treated and untreated varicoceles [26]. The authors identified an increase in clinical pregnancy rates (OR = 1.59, 95% CI, 1.19–2.12) and live birth rates (OR = 2.17, 95% CI, 1.55–3.06) with varicocelectomy prior to ICSI.

Kirby et al. similarly preformed a systematic review and meta-analysis on ART outcome in men with treated and untreated varicoceles [27]. Their study analyzed the data of both oligospermic and azoospermic men. Contradictory to the Esteves et al. study, Kirby et al. did not find statistical significance for pregnancy rates (OR = 1.695, $p = 0.73$), though they did for live birth rates (1.699, $p = 0.042$). However, both studies included the three aforementioned studies and are subject to the same concerns. Additionally, Esteves et al. acknowledge questions as to whether the expense associated with varicocelectomy prior to ART is cost-effective for achieving a live birth as there is a paucity of such published analyses [12].

The Azoospermic Male

Although paternity rate by natural pregnancy after varicocelectomy in azoospermic men is rare, it has been reported that these men may benefit from surgical repair, as 43–55% of them were able to ejaculate sperm after the procedure, in that way sparing the need for testicular sperm extraction before IVF/ICSI [28, 29].

Two retrospectives studies have analyzed the efficacy of varicocelectomy in patients who remained azoospermic before testicular sperm extraction. Inci et al. compared 66 patients who had previously undergone varicocelectomy to 30 patients who had uncorrected varicocele [14]. Male age, male FSH, testicular volume, and female age were similar in both groups. Sperm retrieval was more successful in the treated group (53% vs. 30%, $p = 0.04$); however, the researchers failed to demonstrated improvement on clinical pregnancy and live birth rates (31.4% vs. 22.2%, $p > 0.05$). Haydardedeoglu et al. divided 96 men with azoospermia and grade III varicoceles in two groups (31 treated patients and 65 untreated patients) [13]. They have also demonstrated a higher sperm retrieval rate in the operated group (60.8% vs. 38.5%, $p = 0.01$), but differently from the first study, they were also able to demonstrate higher pregnancy and live birth rates in the first group (74.2% vs. 52.3% and 64.5% vs. 41.5%, respectively, $p < 0.05$). Interestingly, they have included nine patients with genetic abnormalities in the untreated group – four men with Klinefelter's, one man with 47XYY, and four men with Y chromosome microdeletions. Although all had a successful sperm retrieval, three had fertilization failure and one had a negative pregnancy test.

One meta-analysis, compiling the data reported above with azoospermic and non-azoospermic patients, has demonstrated that varicocele repair significantly improved pregnancy rates (OR 1.760, 95% CI 1.139–2.720, $p = 0.0109$) and live birth rates (OR 1.761, 95% CI 1.223–2.537, $p = 0.0024$) [27]. Nevertheless, owing to the obvious lack of randomized controlled trials, no significant correlation can be addressed in this matter, as only observational studies have been designed, despite being considered to be of inferior quality, according to the principles of evidence-based medicine.

Lee et al. constructed a decision analysis for men with non-obstructive azoospermia associated with varicocele [30]. The authors estimated the per live delivery economic impact of varicocelectomy versus immediate microsurgical TESE, utilizing both direct and indirect cost elements (i.e., complications, lost productivity, multiple gestation pregnancies). The decision analysis supported microsurgical TESE over varicocelectomy ($69,731 vs. $79,576, respectively). While the authors acknowledge concerns over publication bias, variability in patient-specific health insurance coverage, and lack of consideration for potential downstream costs of raising children conceived by ART, they suggest that their findings, at minimum, provide a "structured analysis of relevant factors in assisted reproduction."

Conclusion

Though it has been widely investigated, the current published literature contains methodological heterogeneity and does not consistently acknowledge significant possible confounding factors. Each couple ought to be counseled on the possible improvement of semen parameters and reproductive outcomes with varicocelectomy prior to assisted reproduction, but broad applicability should not be undertaken for the non-azoospermic or azoospermic male. Well-designed, randomized controlled trials are needed to appropriately answer the question of whether a varicocele should be repaired prior to assisted reproduction.

Review Criteria

An extensive search of studies evaluating the outcomes of intrauterine insemination and in vitro fertilization, including intracytoplasmic sperm injection in couples with a clinically significant varicocele either with or without repair, was performed using PubMed and MEDLINE. The search was based on keywords: "varicocele repair," "varicocelectomy," "varicocele ligation," "varicocele correction," "varicocele treatment," "assisted reproduction," "intrauterine insemination," "in vitro fertilization," or "intracytoplasmic sperm injection." Abstracts solely published from conferences or meetings were not included in compiling this manuscript.

Multiple Choice Questions and Answers

1. In the setting of female factor infertility with a male partner having normal semen parameters and a varicocele, varicocelectomy is:
   (a) **Not indicated**.
   (b) Indicated prior to female factor treatment.
   (c) Indicated if the varicocele is bilateral.
   (d) Indicated if the varicocele is radiographically identified.
2. The published literature on varicocele repair prior to IUI is limited by:
   (a) Female age reporting.
   (b) Induction protocols.
   (c) Small study populations.
   (d) **All of the above**.
3. Recent meta-analyses have demonstrated improved pregnancy rates with varicocelectomy prior to IVF/ICSI. These findings should be:
   (a) Cited as strong evidence for repair prior to ART.
   (b) **Interpreted with caution given the limitations of the studies included in the meta-analysis**.
   (c) Disregarded as they are not prospective randomized, controlled studies.
   (d) Cited as strong evidence against repair prior to ART.
4. In the azoospermic male with a clinical varicocele, the most cost-effective course is:
   (a) **Immediate microsurgical testicular sperm extraction with IVF**.
   (b) Varicocelectomy followed by re-evaluation for sperm in the ejaculate and possible ART.
   (c) Three months of selective estrogen receptor modulator (SERM) therapy followed by re-evaluation for sperm in the ejaculate and possible ART.
   (d) Varicocelectomy followed by SERM therapy.
5. When considering whether a couple should proceed with ART or varicocelectomy for male factor infertility:
   (a) Varicocelectomy is always indicated prior to ART.
   (b) Varicocelectomy is indicated if the patient is azoospermic.
   (c) Varicocelectomy is indicated if the patient is oligospermic.
   (d) **Female factors, including age, must be considered prior to determining ART versus varicocelectomy**.

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Pro: Should Varicocele Be Repaired in Azoospermic Infertile Men?

44

Sandro C. Esteves

Key Points

- Treatment in patients with nonobstructive azoospermia and varicocele should aim at increasing the likelihood of (i) sperm return to the ejaculate, thus obviating the need for sperm retrieval, and (ii) sperm retrieval success.
- Surgical repair of clinical varicocele might lead to sperm return to the ejaculate in approximately 40% of men with nonobstructive azoospermia.
- NOA men might achieve natural pregnancies with ejaculated sperm after varicocele repair occasionally.
- The chances of success concerning testicular sperm retrieval and sperm return to the ejaculate postoperatively seem to associate with testicular histopathology results showing residual areas of spermatogenesis.
- Men with NOA and clinical varicocele seeking fertility should undergo genetic screening for Y chromosome microdeletions before considering varicocele repair.
- Varicocele repair should be discussed with men with NOA as a means to increase the likelihood of harvesting sperm from the ejaculate or testis to be used for intracytoplasmic sperm injection (ICSI).
- Among NOA patients with clinical varicocele and a successful sperm recovery – either in the ejaculate or testis – varicocele repair pre-ICSI might lead to better reproductive outcomes.

Introduction

In this chapter, we discuss the reasons why urologists might offer repair of clinical varicoceles to a selected group of men with non-obstructive azoospermia (NOA) seeking fertility. We feel discussion concerning this specific topic is better than argument because an argument is to find out who is right and discussion is to find what is right. The critics say that urologists should not offer varicocele repair to men with NOA because the evidence is not good enough to support such recommendation. Moreover, opponents try to persuade readers to believe that supporters of the "PRO" side want to generalize the recommendation of repair to all affected men. Here,

S. C. Esteves (✉)
ANDROFERT, Andrology & Human Reproduction Clinic, Campinas, SP, Brazil
e-mail: s.esteves@androfert.com.br

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we, first of all, present the existing evidence indicating that varicocele repair might be beneficial to improve spermatogenesis in men with NOA. Then, we will discuss the prognostic factors affecting postoperative outcomes as a means to help readers identify who are the best candidates for varicocele repair.

History of Varicocele Repair in Men with Azoospermia

Let's begin with history, which critics tend to forget. Dr. William Selby Tulloch (1913–1988) was the first to report a varicocele repair to treat infertility [36]. Surprising enough, Dr. Tulloch reported a case of an infertile man with bilateral varicocele and NOA. His patient had testicular biopsy-proven maturation arrest and underwent repair by the Robb technique – a method that approaches the spermatic veins 5 cm above the internal inguinal ring [30]. His patient had sperm return to the ejaculate after varicocele repair, and the patient's wife was able to conceive naturally. Dr. Tulloch's report undoubtedly contributed to the increased awareness of the implications of varicocele in infertility and the potential benefits of its repair [27].

Detrimental Effect of Varicocele on Spermatogenesis

The negative impact of varicocele on spermatogenesis has been extensively documented [1, 2, 4, 14, 20, 28, 31]. The pathophysiology of testicular hypotrophy associated with varicocele includes increased levels of adrenal metabolites in testicular blood, excessive reactive oxygen species (ROS), testicular hypoxia, rupture of the testis-blood barrier with a consequent autoimmune response, and decreased testosterone production by Leydig cells [1, 20, 28]. Furthermore, patients with varicocele have increased scrotal temperature. The human Sertoli cells have to maintain a temperature of 2–3 degrees Celsius lower than core body temperature to support spermatogenesis optimally; exposure to excess heat contributes to ROS generation [34]. Excessive free radicals, in turn, may damage both the DNA and proteins in the nucleus of spermatic and tubular cells, as well as Leydig cells, and induce germ cell apoptosis [14, 17]. Men with low baseline sperm production are particularly vulnerable and may develop azoospermia [19, 32]. Increased intratesticular cadmium concentration and reduced levels of androgens can also contribute to reduction in sperm production [3]. Ultimately, it may be a combination of these factors that result in azoospermia in a subset of affected men.

A Significant Proportion of NOA Men Have Sperm Return to the Ejaculate After Varicocele Repair

Azoospermia is found in 4–13% of men with varicocele [5, 8]. After varicocele repair, it is not uncommon that these men have the return of sperm to ejaculates. A 2016 review systematically evaluated the role of varicocele repair in men with NOA [16]. Sixteen studies accounting for a total of 344 men reported data related to sperm return to the ejaculate post repair of clinical varicoceles. The age and follow-up duration of the treated population were 32.5 years (±2.3) and 12.4 months (±5.5), respectively. The proportion of patients with postoperative return of sperm to the ejaculate was 44% (range, 20.8–55.0%). The mean postoperative sperm count and motility were 1.8 million/mL (SD, 1.6; 95% CI, 0.9–2.7) and 23% (SD, 15%; 95% CI, 12–33%), respectively, and the interval between varicocele repair and appearance of sperm in postoperative ejaculate varied from 4.5 to 11 months.

The results of the above study indicate that a significant proportion of men with NOA who have undergone varicocele repair might benefit from the operation. Postoperative complications after varicocele repair are uncommon, in particular using minimally invasive subinguinal microsurgical varicocele repair or embolization [28]. Varicocele repair is usually performed on an outpatient basis with a quick recovery and minimal time off work [9, 10, 26].

Notwithstanding these observations, opponents argue that the existing reports include few patients and lack methodological rigor as no comparison group of non-treated patients is available. However, it is unlikely that a control group of NOA men with untreated varicocele would benefit from expectant management. Although it might be said that treated men who remain azoospermic had the operation in vain, evidence indicates otherwise as men with repaired varicocele tend to have higher sperm retrieval rates than those with untreated varicocele (see "sperm retrieval"). Naturally, there might be a psychological burden from the disappointment concerning treatment failure; however, this can be alleviated by proper patient counseling and optimal selection of candidates to varicocele repair.

Improvement in Semen Quality After Varicocele Repair in NOA Men Translates into Better Chances of Natural and Assisted Conception

Some authors suggest that even though sperm might return to the ejaculate after varicocele repair, these men will not have usable sperm to avoid sperm retrieval [33]. However, we should not overlook the benefit of the return of sperm to ejaculates after varicocele repair. It not only avoids the need for an invasive procedure for harvesting testicular sperm for intracytoplasmic sperm injection (ICSI) but might also allow achievement of natural pregnancies. In the systematic review mentioned above, 14% of the treated population achieved natural pregnancy after sperm return to the ejaculate [16]. The pooled evidence on natural pregnancy included 88 patients who had sperm in postoperative ejaculates, with a mean follow-up of 12.7 months (range, 6–25 months). We should advise our patients that assisted reproductive technology (ART) will likely be required as the postoperative sperm count is low. However, handling ejaculated sperm in the in vitro fertilization (IVF) laboratory is far easier and less time-consuming than processing testicular specimens [11]. Nonetheless, young couples, in particular those in whom the female partner is ovulatory and with an adequate ovarian reserve, might achieve natural pregnancies with ejaculated sperm, as discussed above. Moreover, ART outcomes using fresh ejaculated sperm seem to be higher than that of with sperm harvested from the seminiferous tubules [12, 25, 37]. In a systematic review, followed by meta-analysis, Kirby et al. evaluated the impact of varicocele repair on pregnancy in infertile couples undergoing ART wherein the male partner had oligozoospermia or azoospermia and a coexistent varicocele [24]. The pooled estimated effect from a total of 1241 patients and seven studies indicated that live birth rates were increased when the varicocele had been treated (OR = 1.71, 95% CI, 1.2–2.5, $P = 0.002$).

Taken together, the above data indicate that varicocele treatment in couples presenting with severe male factor infertility, including NOA, can lead to significantly better chances to achieve pregnancy. Therefore, not only sperm return to the ejaculate but also the higher success rate in surgical sperm retrieval are the elements to discuss with the affected patients.

Sperm Retrieval Rates and Pregnancy Outcomes in ART Are Higher in Men with NOA and a Coexistent Treated Varicocele

Naturally, couples presenting with infertility wish to have a healthy live birth. It thus seems crucial to show evidence that varicocele repair in men with NOA might result in significantly better odds to achieve this goal. In the 2016 systematic review mentioned above, three studies provided surgical sperm retrieval (SR) data between treated and untreated men with varicocele and NOA and therefore were meta-analyzed [16]. The observed pooled effect size involving 400 patients indicated that SR success was significantly higher in the treated varicocele group than in the untreated one (OR, 2.6; 95% CI, 1.7–4.1; $I^2 = 0\%$; $P < 0.0001$). The time interval between varicocele repair and SR was 10.8 months (SD, 11.1; range, 3–23.6 months). Two studies involving 140 couples in total reported data on preg-

nancy by ICSI using testicular sperm harvested from the seminiferous tubules [21, 23]. These studies included a control group of men with NOA and untreated varicocele for comparison. The estimated pooled increase in the odds of achieving a clinical pregnancy and a live birth by ICSI using testicular sperm from treated men were 2.2 (95% CI OR, 0.99–4.83; $P = 0.05$; $I^2 = 0\%$) and 2.1 (95% CI OR, 0.92–4.65; $P = 0.08$; $I^2 = 0\%$), respectively [16]. The results discussed above were corroborated by the Kirby et al. meta-analysis, which showed that SR rates (OR = 2.5) and pregnancy rates (OR = 2.3) were significantly higher in persistently azoospermic men after varicocele repair [24].

Collectively, despite the limited data and the obvious need for further research, the existing evidence indicates that azoospermic patients with clinical varicocele who undergo varicocele repair might experience improved SR rates and pregnancy rates with ICSI. The question urologists and health care practitioners providing care to infertile men with NOA and a coexistent varicocele should ask themselves is why to deprive these men the opportunity for improvement.

Predictive Factors for Sperm Recovery After Varicocele Repair in Men with NOA

In a 2017 study evaluating the role of predictive factors for sperm recovery, patient age, testicular volume, varicocele grade, levels of FSH, LH, testosterone, and estradiol were not significantly different between patients who had sperm either in the ejaculate or in the testicle after varicocele repair [35]. In our 2016 review mentioned above, five of the included studies analyzed the association between varicocele grade and the return of sperm to ejaculates [16]. The rates of sperm return were 7.7% (1/13) in patients with grade 1 varicocele, 25.8% (8/31) in those with grade 2, whereas it was 34.3% (11/32) in those with grade 3 varicocele. Despite the apparent increase in sperm return to the ejaculate by repairing larger varicoceles, the pooled effect size estimated did not reach statistical significance (OR, 0.19; 95% CI, 0.02–1.59; $P = 0.09$). Since this analysis comprised only 76 patients, further research might help to answer the question of whether or not varicocele grade affects the odds of having sperm back into the ejaculate after varicocele repair.

On the other hand, testicular histology results can help in predicting the chances of sperm return to the ejaculate [22]. In our 2016 systematic review, eight of the included studies reported data from 161 men concerning the return of sperm to the ejaculate based on testicular histopathology results [16]. Hypospermatogenesis, maturation arrest (MA), and Sertoli cell-only (SCO) syndrome were found in 38.5%, 31.7%, and 29.8% of men with NOA subjected to varicocele repair. The rates of sperm return to ejaculates were 56.2% for hypospermatogenesis, 35.3% for MA, and only 9.7% for SCO. The pooled estimates indicated that the odds of sperm return to the ejaculate were significantly higher in men with hypospermatogenesis than MA (OR, 2.35; 95% CI, 1.04–5.29; $P = 0.04$) or SCO (OR, 12.0; 95% CI, 4.34–33.17; $P < 0.001$). Moreover, the odds of sperm return to the ejaculate were higher in men with MA than SCO (OR, 5.09; 95% CI, 1.83–14.10; $P = 0.001$). An earlier systematic review and meta-analysis of 11 cohort studies involving 233 patients with NOA and coexistent clinical varicocele corroborated our results [38]. Approximately 40% of men had a return of sperm to the ejaculate after microsurgical varicocele repair. The mean sperm count is 1.6 million/ml, and 26% of these men were able to impregnate their partners either naturally or through ART. In this study, hypospermatogenesis and MA were significantly more likely to be associated with the presence of sperm in the postoperative ejaculate compared with SCO (OR, 9.4; 95% CI, 3.2–27.3).

However, performing a testicular biopsy in men with NOA for diagnostic purposes is a matter of debate. Firstly, due to testicular heterogeneity, none of the histopathology phenotypes can absolutely predict who may or may not have sperm return to the ejaculate [13, 35]). Secondly, extraction of testicular parenchyma might inflict additional harm as men with NOA usually have small testes and abnormal androgen production.

Lastly, biopsied specimens might contain mature spermatozoa that would be wasted after fixation and staining. Thus, despite the clinical utility of histopathology data for counseling, caution should be applied to recommend it for men with NOA routinely. If one opts to do so, it is advisable to also performing a wet examination of the extracted specimen and freezing the testicular parenchyma in case sperm are identified.

Genetic screening of the long arm of the Y chromosome has proved to be of value not only to identify those patients for whom Yq microdeletions cause NOA but also to predict the chances of retrieving sperm. In practical terms, finding a microdeletion within the AZFa or AZFb region means that the odds of harvesting sperm from the seminiferous tubules are virtually nil, irrespective of the method used for sperm acquisition [15]. By contrast, patients with isolated AZFc microdeletions often harbor residual spermatogenesis, with SR success ranging from 50% to 70%. It is therefore advisable to screen men with NOA and coexistent varicocele for Y chromosome microdeletions (YCMD). The finding of a varicocele in men with AZF microdeletions and NOA might be coincidental, thus meaning that spermatogenic failure is due to the underlying genetic cause rather than the varicocele [29]. While varicocele repair (and sperm retrieval) is unjustified in men with AZFa or AZFb microdeletions, it remains unclear if repair of palpable varicoceles might offer any benefit for those with AZFc microdeletions. To our knowledge, no study has examined SR success in men with AZFc microdeletions and varicocele. However, the association between varicocele and YCMD in men with NOA seems to be small. A cross-sectional 2018 study involving 51 men with azoospermia and severe oligozoospermia revealed that YCMD was found in less than 4% of patients [7], albeit other investigators report higher frequencies varying from 18% to 23% in Chinese men [6, 18].

At present, it seems sound to screen all men with NOA and a coexistent varicocele seeking fertility for YCMD. Given the existing evidence, men with AZFc microdeletions should be advised to proceed with SR and ICSI. While a testicular biopsy showing hypospermatogenesis or maturation arrest is suggestive of better outcomes, it should be considered only in selected cases and if wet examination and sperm freezing are readily available. Noninvasive seminal plasma markers to predict those men most likely to have sperm return to the ejaculated post varicocele repair are under investigation. These markers, such as micro-RNA-192a levels, might help identify eligible NOA men for varicocele repair [39]. Collectively, it is clear that more research is needed to establish the role of prognostic factors for a better selection of men with NOA to undergo varicocele repair.

Facts Used by Critics to Try to Persuade Infertility Specialists Not to Discuss Repair of Clinical Varicoceles in NOA Men

Critics often use long-winded and eloquent language to state that the recommendation of varicocele repair to men with NOA is not evidence-based. They claim that there is a lack of randomized controlled trials and large cohort studies and add that the current recommendation is based on erroneous assumptions. While it is far too easy to attack the credibility of cohort studies showing a positive association, they also forget the robust evidence examining the pathophysiology of varicocele and its adverse effect on fertility. Furthermore, opponents tend to use emotional language to imply that the burden of a failed operation is too heavy for the affected men to support. Arguments like frustration, costs, recovery time, and complications are often utilized to attack those who want to discuss the potential benefit of varicocele repair to men with NOA.

What these critics want to emphasize, as usual, are the negative aspects only. On the contrary, a balanced discussion should also include the potential positive effect of such a minimally invasive procedure. About 40% of treated men will have the return of sperm to the ejaculate. Those who remain azoospermic seem to have

higher success when subjected to sperm retrieval, and use of sperm from treated men yields better reproductive outcomes. In addition to that, it is important to remember that ICSI failure is common. Multiple ICSI attempts might be required until pregnancy is established. Along these lines, proceeding directly to sperm retrieval would not be at the best interest of the couple at all if an intervention was available that could help these couples to repeat ICSI, if needed, with ejaculated sperm. Although one may argue that some couples will succeed with sperm retrieval and ICSI, we should bear in mind that most men with NOA will not be candidates for repeated sperm retrieval. Although testicular sperm freezing is feasible, ICSI results – in general – favor ejaculated sperm than frozen-thawed sperm in men with NOA. Therefore, having sperm in the ejaculate to allow ICSI without the need to subject the men to sperm retrieval is obviously advantageous, even if success rates were similar with ICSI using ejaculated and surgically retrieved sperm.

Naturally, urologists should discuss with their patients with NOA and a coexistent clinical varicocele the pros and cons when counseling about varicocele repair. Use of information from genetic screening and testicular histopathology (if available) might help patients to make informed decisions. It is hard to believe that any urologist with proper training in male infertility would suggest varicocele repair for all men with NOA without considering other prognostic factors.

Acting Responsibly: A Proposed Algorithm for the Clinical Management of Men with NOA

Nonobstructive azoospermia is the most severe male infertility condition, and the coexistence of a clinical varicocele in such men is not uncommon. Overall, 50% of men with NOA, including those with varicocele, have residual sperm production within their dysfunctional testes. Although NOA due to spermatogenic failure is an irreversible condition, the affected men might have their chances of having sperm harvested – from the ejaculate or testicle – optimized. Consequently, their chances of biological fatherhood might also be improved. A series of steps that includes the differential diagnosis of azoospermia, genetic testing and counseling, identification of those who could benefit from medical and surgical interventions before SR, application of the best method to retrieve spermatozoa, and the use of state-of-the-art laboratory techniques are vital to achieving these goals (Fig. 44.1). It is out of the scope of this chapter to discuss these aspects in detail, but a comprehensive discussion on the matter concerned can be found elsewhere [15]. A coordinated multidisciplinary effort involving

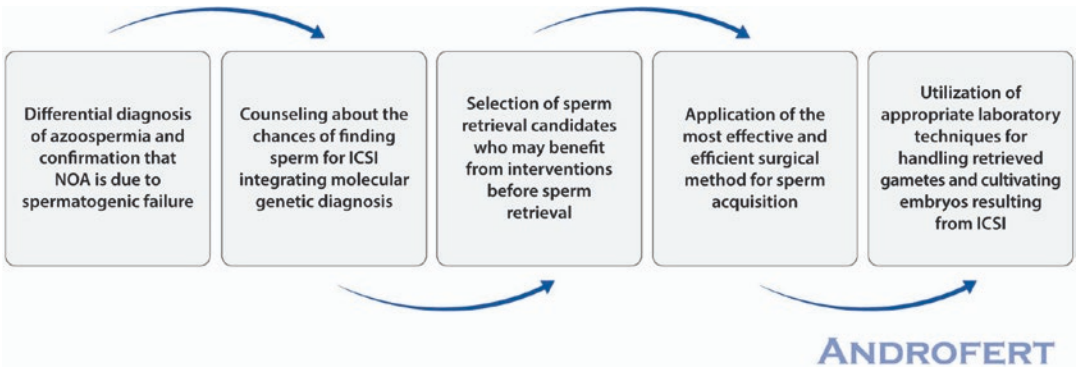

Fig. 44.1 Proposed algorithm for a step-by-step clinical approach in the management of infertile men with nonobstructive azoospermia. (Reprinted from Esteves [15]. With permission from Wolters Kluwer Medknow Publications)

reproductive urologists, geneticists, reproductive endocrinologists, and embryologists are critical to offering these men the best possible chance of having their biological children.

A Glimpse into the Future

Research focusing on prognostic factors and noninvasive markers to better select eligible NOA men for varicocele repair is warranted. Moreover, further comparative studies including the critical reproductive outcomes might allow better judgment of the likely clinical impact of varicocele repair on this subset of men. While waiting for new information that might fill the existing gaps in knowledge, clinicians should exercise their best judgment and apply the evidence that is available to discuss transparently with their patients with NOA and coexistent palpable varicocele the benefits and limitations of varicocele repair.

Review Criteria

An extensive search of studies examining the relationship between varicocele and nonobstructive azoospermia was performed using search engines such as ScienceDirect and PubMed. The end date for these searches was September 2018. The overall strategy for study identification and data extraction was based on the following keywords: "varicocele," "azoospermia," "sperm retrieval," "varicocele repair," "varicocelectomy," "infertility," "male," "assisted reproductive technology," "intracytoplasmic sperm injection," "semen parameters," "reproductive outcomes," and "pregnancy rate." Articles published in languages other than English were not considered. Data that were published in a conference or meeting proceedings, websites, or books were used to provide conceptual content only.

Conclusions

The recommendation of repairing clinical varicocele in a selected group of NOA men seeking fertility is based on a combination of factors including (i) the detrimental effect of varicocele on spermatogenesis; (ii) evidence indicating that a significant proportion of NOA men have sperm return to the ejaculate after varicocele repair; (iii) evidence showing that improvements in semen quality after varicocele repair in NOA men translates into better chances of natural and assisted conception; (iv) supportive data indicating that sperm retrieval rates and pregnancy outcomes in ART are higher in men with NOA and a coexistent treated varicocele. Further research is required to quantify the role of varicocele repair in NOA men more precisely and to determine the factors which might be used to best select eligible patients for varicocele repair. At present, doctors should apply the evidence that is available and discuss with their patients the potential benefits of having a varicocele repaired as a means to improve the chances of achieving a live birth, either naturally or assisted.

Multiple Choice Questions and Answers

1. Which of the following statements support the recommendation of varicocele repair to selected men with NOA men seeking fertility?
   (a) Documented detrimental effect of varicocele on spermatogenesis.
   (b) Evidence indicating that a significant proportion of NOA men have sperm return to the ejaculate after varicocele repair.
   (c) Evidence showing that improvements in semen quality after varicocele repair in NOA men translates into better chances of natural and assisted conception.
   (d) Data indicating that sperm retrieval rates and pregnancy outcomes in ART are higher in men with NOA and a coexistent treated varicocele.
   (e) **All of the above.**
2. The following evidence-based recommendations can be offered to men with NOA and a coexistent varicocele seeking fertility, except:
   (a) Y chromosome microdeletion screening.
   (b) **Empirical medical therapy.**

(c) Diagnostic testicular biopsy.
(d) Microsurgical varicocele repair.

3. Which of the following prognostic factors are associated with an increase in the likelihood of sperm return to the ejaculate after varicocele repair in men with NOA?
   (a) Presence of a Yq AZFa microdeletion.
   (b) Presence of a Yq AZFb microdeletion.
   (c) Karyotype showing 47,XXY.
   (d) **Presence of areas of active spermatogenesis on testicular histopathology.**
4. Which of the following has the highest likelihood of occurrence after varicocele repair in a man with NOA?
   (a) Testicular atrophy.
   (b) Natural pregnancy.
   (c) **Sperm return to the ejaculate and improvement in sperm retrieval success.**
   (d) Hypogonadism.
5. Concerning repairing clinical varicoceles in men with NOA, which of the following statements are supported by the existing published evidence?
   (a) Varicocele repair improves the chances of sperm return to the ejaculate in men with signs with active spermatogenesis.
   (b) Varicocele repair can increase the likelihood of sperm retrieval success in men who remain azoospermic after varicocelectomy.
   (c) Varicocele repair can improve pregnancy rates with ICSI.
   (d) **All of the above.**

Conflict of Interest The author has nothing to disclose.

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Con: Should Varicocele Be Repaired in Azoospermic Infertile Men?

45

Peter T. K. Chan

Key Points

- Repair of clinical varicocele may improve sperm recovery and retrieval rate in only a minority and selected group of men with non-obstructive azoospermia.
- The odds of improvement appeared to correlate to the presence of spermatogenic activities as seen in testicular biopsy.
- The benefits and limitations of varicocele repair must be clearly disclosed to men with NOA and varicoceles to allow them to decide whether they should undergo varicocele repair or directly to sperm retrieval for ICSI.
- Unfortunately, there is no good evidence supporting that such an improvement after varicocele repair could lead to better reproductive outcomes such as live birth rate with assisted reproduction in NOA men.
- Varicocele repair should not be offered to all NOA men until further prospective comparative studies are conducted to clearly demonstrate the efficacy of varicocele repair in improving live birth rate.

Introduction

In this debate, we will present the view why repair of clinical varicoceles should not be routinely performed for all men with non-obstructive azoospermia (NOA). We will focus this discussion in the context of infertility and exclude the other indications of varicoceles repair including pain/discomfort, aesthetic improvement, and correction/reduction of low testosterone risk.

As eloquently presented by Sandro C. Esteves in Chap. 44, there are several reasons why many reproductive urologists come to the conclusion that when counselling non-obstructive azoospermic men, correction of clinical varicoceles first to optimize testicular function should be recommended. This approach unfortunately is not supported by evidence available in the current literature. This incorrect view was a result of a combination of faulty assumptions and inappropriate extrapolations and generalization of the outcomes of varicocele repair to all men with NOA.

Only a Minority of NOA Men Have Sperm Return to the Ejaculate Post-varicocele Repair

To begin with, let's consider the success rates of having sperm returned to ejaculate from men with NOA after repair of clinical varicoceles. The mean success rate in the literatures was 36%

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P. T. K. Chan (✉)
Department of Urology, McGill University Health Centre, Montreal, QC, Canada

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(21–56) (see reviews by [8, 28]). It would be too easy to attack the credibility of these studies by noting their general small sample sizes, retrospective nature, and lack of a proper comparison group. But even if one accepts the absolute effect of varicocele repair noted in these studies, it still means that the mean failure rate of such an intervention is 64% (45–79). It is therefore fair to say that the majority of these men with NOA underwent varicocele repair gain little but bear the risk and cost of the procedure, time off work for the procedure and recovery, and, more importantly, the emotional burden from the disappointment and frustration of treatment failure. Like all other men with NOA, those who remain azoospermic after a failed varicocele repair would be counselled to undergo surgical sperm retrieval for ICSI – arguably what should have happened in the first place for these majority of NOA men with varicoceles.

Can we do better than that? Yes! Recent advances in molecular genetic testing demonstrated that global gene expression on the transcriptome of testicular tissue analyzed using next-generation sequencing could reveal a number of cell cycle-related genes that were upregulated and several antioxidant genes that were downregulated in NOA men who had sperm recovery post varicocele repair [30]. This assessment allows prediction of sperm recovery in subgroups of NOA men undergoing repair of varicoceles. Further, micro-RNA-192a levels in seminal plasma and testicular tissue evaluated by quantitative real-time polymerase chain reaction appeared to be a potential marker to distinguish NOA men with or without sperm recovery post varicocele repair [38]. Taken together, there are new ways, possibly not requiring an invasive testicular biopsy, being developed allowing us to identify those NOA men with clinical varicocele that are more likely to have sperm returned to ejaculate and potentially avoid unnecessary varicocele repair for the majority of NOA men.

Even if next-generation sequencing and micro-RNA analysis are not readily available in your neighborhood clinical laboratories, various studies [1, 2, 10, 17, 35] have indicated that a simple history assessment from a testis biopsy could also indicate the chance of sperm recovery in NOA men post varicocele repair. The odds of sperm recovery for the three most common histologies are 45% for hypospermatogenesis; 28% for maturation arrest, with significantly lower rate for early maturation arrest [35, 36]; and 14% for Sertoli cell-only pattern [28]. Thus, though testicular histology could not absolutely predict or exclude those who may not have sperm recovery in the ejaculate, proper use of predictive values derivable from these data for counselling may potentially minimize unnecessary varicocele repair in the majority of these NOA men with low odds of sperm recovery and advise them to proceed with surgical sperm retrieval for ICSI.

Why was this information, which has been described by numerous studies for over a decade, not used routinely when counselling patients? Some investigators noted the “invasiveness” of a testis biopsy and the associated risks of removing and wasting precious mature spermatozoa that could be used for reproduction [6, 13]. Others indicated that analysis of a single testicular biopsy is prone to errors from poor tissue fixation and handling, discrepancies in pathology reporting (e.g., reporting the predominant rather than the most advanced spermatogenic pattern), and sampling error in some NOA men with heterogenous pattern of testicular histology [2, 5, 34]. Clearly there is a need to properly conduct well-designed prospective comparative studies to establish the predictive values of testicular histology in this context. Until such studies are conducted, it is only fair for clinicians to fully disclose in an unbiased manner the currently available data when counselling NOA men with clinical varicoceles whether they should undergo varicocele repair rather than offering the intervention to all of them.

Does Varicocele Repair for NOA Men Really Improve the Couples’ Odd of Having Live Birth?

Presumably advocates for repairing all varicoceles in NOA men were under the assumption that improvement in sperm recovery rate after

varicocele repair represents an "upgrade" in their fertility status and will lead to improved subsequent reproductive outcomes. But further questions remain to be answered with regard to the putative positive impact of varicocele repair in NOA men. First, for those who have sperm returned to ejaculate post-varicocele repair, do they have better reproductive outcomes due directly to the repair? It should be noted that even for these NOA men with sperm returned to ejaculate post-varicocele repair, they rarely have adequate amounts to avoid TESE [29] to obtain usable sperm for reproduction. Further, some authors indicated that the time required post-varicocele repair to see sperm returned to the ejaculate may take over 12 months [24, 36] which may represent a significant delay that many infertile couples cannot afford due to such factors as advanced female age and low ovarian reserves. More importantly, even among those who have sperm returned to the ejaculate, some may "relapse" to azoospermia [1, 20, 24, 36], leading to the speculation if the observation of sperm returning to ejaculate merely represents a fluctuation of baseline spermatogenic activity rather than a true improvement of reproductive status from the varicocele repair. In any case, several groups did report a few cases (13.6% estimated by [9]) of unassisted conception [10, 11, 18, 20, 22, 24, 25, 33, 35, 36]. Though, for obvious reasons, there were with no comparison group, these rare cases represented improvement in the reproductive outcomes. But for the majority of these NOA men with sperm returned to ejaculate who still require ICSI post-varicocele repair, do they have better outcomes than those NOA men who did not undergo varicocele repair and required surgical sperm retrieval for ICSI? A few studies [11, 17, 18, 22, 25] have reported pregnancy outcomes (estimated to be 18.9% by [9]) with ICSI using recovered sperm post-varicocele repair. However, no comparison was made with ICSI outcomes using surgically retrieved sperm from NOA men without varicocele repair. In other words, there is no evidence demonstrating improved reproductive outcomes after varicocele repair among NOA men who have sperm returned to the ejaculate.

Further, for the majority of these NOA men after varicocele repair that required subsequent surgical sperm extraction for ICSI, do they have better reproductive outcomes than NOA men with varicocele who do not have repair and proceed directly to sperm extraction for ICSI? Sperm retrieval rate was significantly higher in NOA men who had prior varicocele repair compared to those who did not undergo varicocele repair [9, 12, 14, 37], though other conflicting data exist [29]. With regard to pregnancy and live birth rates, there were only two available studies [12, 14] with comparative data but showing conflicting results on the benefits of varicocele repair. Two meta-analyses of these studies [9, 19] have both concluded that the quality of evidence is too poor to demonstrate any significant improvement on either the pregnancy or live birth rate with ICSI among men with NOA that had undergone varicocele repair. Again, there is no evidence demonstrating improved pregnancy or live birth rates after varicocele repair in NOA men undergoing sperm retrieval for ICSI.

With over two dozen studies demonstrating some benefits of repairing varicoceles in men with NOA, it is puzzling why only two studies have included data on the most relevant reproductive outcomes, namely, pregnancy and live births. How much more difficult is it to conduct follow-up studies on these subjects (who were already identified and recruited) to record the outcomes prospectively or, even simpler, to retrospectively look up the reproductive outcomes? In clinical research we always have to be aware of the impact of publication bias – studies with positive or favorable outcomes are more likely to be completed, written up, and published than those with negative or unfavorable outcomes. Could it be the case that investigators who claimed a positive impact of varicocele repair in NOA men actually have conducted follow-up studies and failed to demonstrate better reproductive outcomes and therefore chose not to publish the results? This is of course a mere speculation but is worth readers' consideration on why for over a decade we have little data to support the benefits on the reproductive outcomes of varicocele repair for NOA men.

Factors that Make Reproductive Urologists Wrongly Advocating Repairing Clinical Varicoceles in All NOA Men

Let us not forget that when a couple present with infertility (severe male factor infertility in this discussion), their goal of treatment is one thing and one thing only: to have a healthy live birth. It thus seems obvious enough that any treatment recommendable should at least unequivocally lead to significantly better odds to achieve this goal. But somehow investigators and clinicians lead these couples to believe that any management strategies should be equally recommendable if they could provide improved success in any of the middle steps or processes without actually leading to achievement of the ultimate goal of having a live birth. They do so by citing literature with data showing, for example, increased testicular volume, more optimal hormonal profile such as higher serum testosterone, sperm returning to ejaculate, and higher success rate in surgical sperm retrieval. Further, if data is available, they would quote better reproductive outcomes in fertilization rate, embryo quality, implantation rate, and pregnancy rate and justify to their patients that they are of equal importance as live birth rate. They would convince themselves and their patients that these surrogate outcomes are better measurement as they (e.g., testis volume, hormonal profile, sperm retrieval rate) are unconfounded by the variations in the female fertility status and are more readily measured than live birth rates, which takes longer follow-up periods to obtain (yet it usually only takes 10 months in each pregnancy to get to live birth!).

These investigators use the following logic: you need good testes (testicular volume) that have good function (testosterone production) to make sperm (sperm return to ejaculate or higher sperm retrieval rate) that can fertilize an egg (fertilization rate) to make good embryo (embryo quality) that can be successfully implanted into the wife's uterus (implantation rate) and stay there to allow her to be pregnant (pregnancy rate) in order to have a baby (live birth rate). So improvement in any of the middle steps along will logically lead to better chance to have a live birth. Unfortunately, in the presence of pathological conditions (i.e., infertility of various etiologies), this logic does not necessarily hold true. In other words, pathological conditions may trump the chance of live birth so that no matter how much you improve the success of any middle steps, it will increase only your hope but not the actual odds of having a live birth. Hence, the risks, costs, and time spent repeatedly on these interventions are in vain, despite improvement in the success rates of any of the surrogate outcomes of the intermediate steps. Essentially, recommending an intervention without actually having proof that it can improve live birth rate is tantamount to practicing non-evidence-based medicine. Most importantly, the couple may have to bear further risks, cost, time, and often emotional burden of failure to undergo repeated trials of assisted reproduction based on their false belief that they now have better chance to have live birth.

There are further reasons and motivational factors that led these advocates to their position. The effectiveness of varicocele repair in reproductive medicine has long been a subject of debate. With the lack of adequate number of large-scale randomized controlled trials (RCTs) and the presence of a number of biased comparative studies (see reviews by [15, 32]), experts have struggled to establish the indication of clinical varicocele repair. Thus, over the years, we have witnessed evolutions and variations of recommendations on varicocele repair from various reputable medical societies (see reviews by [26, 31]) including the American Urological Association [4], American Society of Reproductive Medicine [3], European Association of Urology [16], and National Institute for Health and Care Excellence [23]. Currently, most of these societies support the view that there are overall benefits of clinical varicocele repair for improving fertility status in men. It seems therefore logical to extrapolate the benefits of varicocele repair to all scenarios of male infertility, including those with NOA. This extrapolation however is irrational considering that the overall positive impact of varicocele repair is far from

being sizable or robust enough for this generalization. In other words, in any given subgroup of patients with varicoceles, there may or may not be similar benefits as seen in all infertile men at large. Yet, with the endorsement as seen in the practice guidelines of the various reputable academic societies, along with having a number of renowned reproductive urologists from large centers noting treatment benefits, it is so easy for advocates to promote repair of all clinical varicoceles to NOA men.

Another important consideration is the "hammer and nail phenomenon" – if you are a hammer, everything looks like a nail. Surgical management, be it varicocele repair, sperm retrieval, or reconstruction of obstruction of the reproductive tract, is what these reproductive urologist do best in male infertility management. Therefore, when facing an infertile couple with the diagnosis of NOA (the most severe form of male infertility to manage) who happen to have concomitant varicoceles (the most common "correctable" male factor infertility), it is only natural for these clinicians to offer a varicocele repair. After all, varicocele repair is considered minimally invasive and is generally covered by most insurance. The recommendation to proceed first with varicocele repair for NOA men has nothing to lose and all to gain.

Even if the repair fails to yield return of ejaculated sperm, the couple is likely prepared to proceed to ICSI with surgical sperm retrieval – yet another surgery that can be offered by the same friendly reproductive urologist. Would the couple question their decision? Imagine an average infertile couple who were shocked to learn that the husband has azoospermia and were told that after a straightforward intervention (varicocele repair), there is a 36% chance of making sperm return to the ejaculate, and, even if unsuccessful, the chance of surgically retrieving sperm for ICSI will increase [12, 14]. It is comprehensible that most infertile couples, if not all, would accept the intervention. Keep in mind, delivery rate of assisted reproductive technology is in the range of 20% on average, and yet over a million cycles per year worldwide are accepted by infertile couples to go through [21]. Taken together, with all these circumstantial factors, promoting varicocele repair to NOA men face little challenge.

Looking Forward

How should reproductive urologists proceed forward responsibly? No doubt more investigations are required. While it is noble to propose more large-scale multi-center RCTs to study this question, we would all agree that, like many research questions in clinical medicine, RCT is highly unlikely to happen. Instead, conducting prospective comparative studies with long-term follow-up to obtain reproductive outcomes including live birth rate is far more feasible to accomplish.

To begin with, one should better characterize the recruited NOA men with clinical varicoceles by first performing multiple standardized semen analyses with centrifugation to search for rare ejaculated sperm (Fig. 45.1), which would indicate at least some levels of complete spermatogenic activities that could benefit from varicocele repair. Other characteristics that suggest existing (currently or previously) spermatogenic activities include the presence of ejaculated sperm in remote semen analyses, previous testicular biopsy, and previous proven fecundity (natural or assisted). Though most reported series excluded men with genetic anomalies [1, 2, 14, 18, 24, 30, 34, 36–38], studies have stated that the two most common genetic anomalies in male infertility, namely, non-mosaic Klinefelter's syndrome and Y-chromosome microdeletion in the AZFc region, have sperm retrievable surgically at over 50% [7]. Thus, NOA men with these genetic diagnoses may also be candidate to undergo varicocele repair. Other genetic diagnoses such as 45XO, 46XX male, and Y-chromosome microdeletion involving the AZFa or complete AZFb regions, on the other hand, should be excluded [7, 27] as they may not have foci of spermatogenesis that can benefit from repair of their clinical varicocele for fertility status improvement.

For all other NOA men with clinical varicoceles, including those with a history of mumps orchitis, cancer therapies, cryptorchidism, and idiopathic NOA, one should consider obtaining

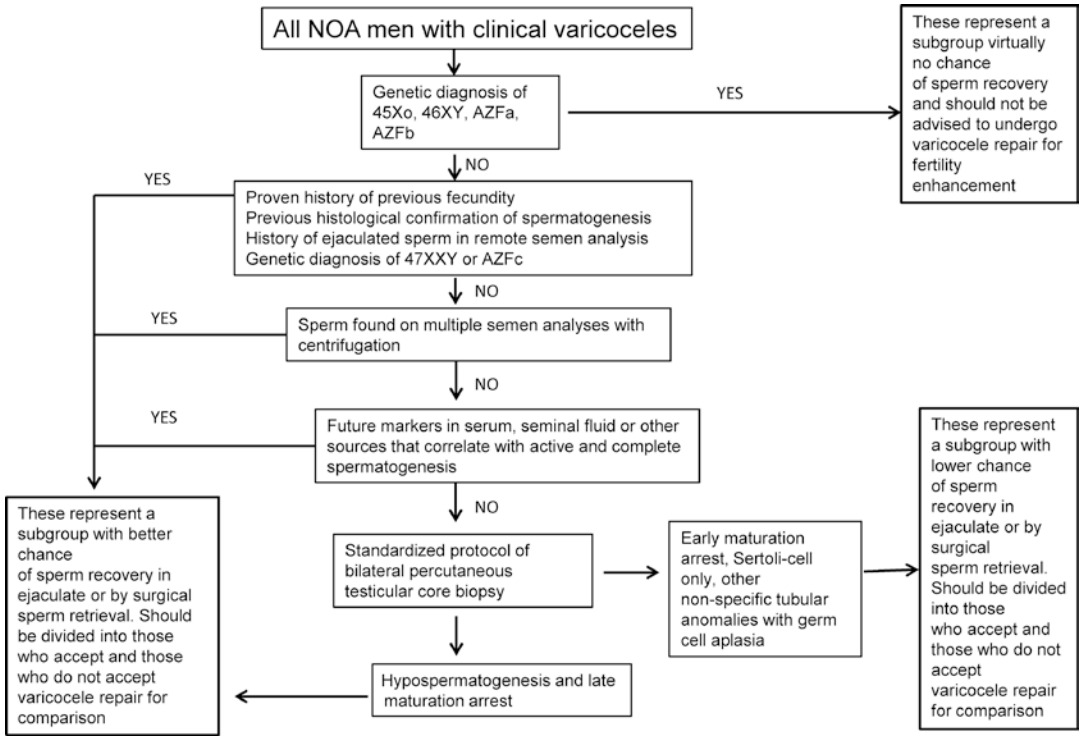

Fig. 45.1 Schematics of a comparative study to evaluate the impact of varicocele repair in NOA men on improving reproductive outcomes

bilateral percutaneous core diagnostic biopsy of the testes under local anesthesia with standardized tissue handling, fixation protocol, and pathological interpretation (e.g., reporting the number of tubules, percentage of predominant patterns including most advanced spermatogenic subtype). With their further development, new molecular genetic strategies, as described earlier in this chapter, may have a role in the near future in correlating the chance of sperm recovery in NOA men post-varicocele repair.

After this characterization of the NOA men, longitudinal follow-up should be performed to compare the outcomes (again, including live birth rate) for those who accept to under varicocele repair versus those who do not accept. The advantage of this protocol is threefold: (1) it standardizes the characterization of NOA men base on the odds of having some levels of complete spermatogenesis prior to varicocele repair; (2) it minimizes the number of NOA men requiring testicular biopsy; and (3) it standardizes the reporting and the procedure of testicular biopsy by using a minimally invasive percutaneous approach that could maintain similar sampling error rate for all subjects.

Conclusion

The recommendation of repairing all clinical varicocele in NOA men appeared to base on a combination of factors including (1) unjustified extrapolation of the recommendation from most clinical practice guidelines that correction of clinical varicoceles will lead to an overall improvement in male fertility status, (2) unsupported view that improving sperm recovery or sperm retrieval rates will ultimately lead to improved live birth rate, and (3) ease of access to and the minimal invasiveness of varicocele repair. Currently, there are approaches already available (e.g., testicular biopsy) or in development (e.g., using molecular genetic methodologies) to allow some prediction of which NOA men with clinical varicoceles that may have better

odds to have sperm recovery. Proper application of this information may potentially avoid unnecessary varicocele repair in NOA men with low odds for sperm recovery. Besides providing such information in an unbiased manner to counsel infertile couples diagnosed with NOA and clinical varicoceles, investigators from large centers are encouraged to conduct prospective comparative studies to properly stratify these NOA men based on pretreatment clinical characteristics to better determine the odds of varicocele repair in improving reproductive outcomes including the live birth rate.

Review Criteria

An extensive search of studies examining the relationship between varicocele and nonobstructive azoospermia was performed using search engines such as ScienceDirect, Ovid, Google Scholar, PubMed, and MEDLINE. The end date for these searches was August 2018. The overall strategy for study identification and data extraction was based on the following keywords: "varicocele," "varicocele repair," "reproductive outcomes," "assisted reproduction," "varicocelectomy," "infertility," "semen parameters," and "pregnancy rate." Articles published in languages other than English were also considered. Data that were published in conference or meeting proceedings, websites, or books were used to provide conceptual content.

Multiple Choice Questions and Answers

1. Which one of the following criteria best correlates with the odds of having sperm returned to the ejaculate after repair of clinical varicocele in men with non-obstructive azoospermia?
   (a) **Histology of testis**
   (b) Serum FSH level
   (c) Testicular volume
   (d) Age
2. Testicular biopsy before varicocele repair in NOA men has been associated with all of the following adverse events *except*:
   (a) Removal and wasting of mature spermatozoa
   (b) **Reducing the efficacy of varicocele repair**
   (c) Sampling error leading to misdiagnosis
   (d) Testicular injury
3. Which one of the following characteristics in an NOA man with clinical varicoceles will have the best likelihood to have sperm returned to ejaculate after varicocele repair?
   (a) Histology of early maturation arrest
   (b) **Previous failure of ICSI using his testicular sperm**
   (c) Genetic evaluation with a 46XX karyotype
   (d) Genetic evaluation with a Y chromosome microdeletion at AZFb and AZFc regions
4. Which of the following event has the highest likelihood to occur in an NOA man after repair of clinical varicoceles?
   (a) Return of motile sperm to the ejaculate
   (b) Natural pregnancy
   (c) Improvement of live birth rate after ICSI
   (d) **Remain azoospermic**
5. With regard to repairing clinical varicoceles in men with NOA, which of the following is supported by the current evidence in the literature?
   (a) All clinical guidelines recommend repairing varicoceles in men with NOA
   (b) Repairing varicoceles is a cost-effective options to increase live birth rate
   (c) Repairing varicoceles will significantly improve pregnancy rate with ICSI
   (d) **Repairing varicoceles can improve sperm recovery and retrieval rate**

Disclaimer The contents and the opinions presented in this chapter are for the purposes of academic exchange and education only and should not be used in isolation for any clinical decision making without a proper consultation with qualified healthcare professionals.

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Should Varicoceles Be Managed Surgically or Radiographically? (Surgery)

46

J. Abram McBride, Daniel J. Mazur, and Larry I. Lipshultz

Key Points

- While there are technical considerations for choosing a particular approach for varicocele repair, embolization is unique in that it suffers from a significant rate of initial failure to treat.
- Various approaches to varicocele repair have demonstrated improvements in clinical outcome measurements; however, it is difficult to compare these techniques given the lack of quality, prospective randomized trials.
- Surgery and radiographic approaches offer favorable complication profiles, but only microscopic inguinal or subinguinal varicocelectomy results in the lowest recurrence and complication rates.
- Only the intraoperative use of an operative microscope and microvascular Doppler has been shown to maximally reduce complication rates compared to alternative surgical approaches.
- Recurrent varicoceles may be treated with either surgery or radiographic techniques with equivalent outcomes, although radiographic approaches may be preferred if the recurrence is bilateral or after previous surgery.

Introduction

The varicocele was initially described by Celsus in the first century AD, but effective treatments were not developed until the introduction of the inguinal varicocelectomy by Narath in 1898 [1]. In 1949, Palomo introduced an alternative—the high retroperitoneal ligation approach [2]. But the perception of varicoceles as innocuous conditions remained until 1955 when Tulloch et al. reversed a case of azoospermia by performing a bilateral high retroperitoneal ligation, establishing a new role for varicocelectomy in the treatment of male infertility [3].

In 1978, Lima et al. introduced the first radiographic approach by performing percutaneous transvenous left spermatic vein occlusion [4]. Meanwhile, microsurgical technology was rapidly developing, and some suggested microscopic enhancement during varicocelectomy to prevent inadvertent ligation of the testicular artery [5]. However, the first reported use of microscopic assistance during inguinal

J. A. McBride · D. J. Mazur · L. I. Lipshultz (✉)
Scott Department of Urology, Baylor College of Medicine, Houston, TX, USA
e-mail: larryl@bcm.edu

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varicocelectomy was not until 1985, the same year Marmar et al. published the first series utilizing a subinguinal microsurgical technique [6]. Finally, with the advent of laparoscopic surgery in the 1990s, the high retroperitoneal approach became preferentially performed laparoscopically [7].

While radiographic embolization remains in the armamentarium for varicocele treatment, surgical approaches, namely, microscopic inguinal and subinguinal techniques, are considered the standard of care. In this chapter, we will review why surgery is preferred to radiographic approaches by discussing technical considerations and comparing clinical outcomes, complications, recurrences, and cost-effectiveness, highlighting specific situations where radiographic techniques can play a unique role in contrast to surgery.

Technical Considerations

There are technical considerations in performing the various varicocelectomy techniques, which can affect the outcome of the treatment and the preference of one procedure over another. This includes the possibility of an inability to treat with embolization, the optimal surgical equipment, anesthetic concerns, and special considerations in the adolescent and obese populations.

Failure to Treat

One of the most important technical considerations is the possibility of a failure to treat during the initial treatment session with radiographic embolization. This occurs mainly from failure to access the internal spermatic vein. While this can be highly patient and operator dependent, a meta-analysis found a mean failure to treat of 13.05% (range 4.2–27.3%) among five studies [8]. Due to anatomical factors, a failure to treat is significantly more common on the right than the left side, with one series demonstrating an 18.9% failure rate for right-sided embolization attempts vs. 3.2% for left [9].

The failure to treat phenomenon with radiographic approaches is unreported with surgery and should be taken into consideration when counseling patients on the best approach to initial treatment of their varicocele.

Finally, not all centers may have the facilities required for embolization. The necessary microcatheter, specific embolic agents or devices, and specially trained interventional radiologists with experience in internal spermatic vein occlusion procedures will all be needed.

Surgical Equipment

The routine use of the operative microscope and microsurgical techniques during inguinal or subinguinal varicocelectomy is the surgical standard of care. While some surgeons perform these procedures macroscopically or loupe-assisted, several studies have shown that these approaches have higher complication and recurrence rates [10–12].

Similarly, the use of intraoperative Doppler assistance has outperformed non-Doppler-assisted, microsurgical subinguinal varicocelectomy with significantly more spermatic arteries spared (1.9 vs. 1.3), spermatic veins ligated (7.8 vs. 7.0), and shorter operative time (41.9 vs. 52.7 min) in the Doppler-assisted group [13]. These authors prefer to use the 20 megahertz microvascular Doppler system by Vascular Technology™ (VTI) during all varicocelectomy cases. The 1.5 mm tip is significantly smaller than alternative Doppler probes and is well suited to identify the small vessels of the spermatic cord. Indeed, the standardly available, larger Doppler probes carry a higher risk of misidentifying spermatic arteries, leading to possible arterial injury.

As such, when choosing to perform an inguinal or subinguinal varicocelectomy, one should use an operative microscope, microsurgical techniques, and a microvascular Doppler to maximize success and minimize complications. If this equipment is not available or the surgeon is uncomfortable with its use, then one may consider embolization as a viable alternative treatment if a skilled interventional radiologist is available.

The Morbidly Obese Patient

While there is limited published data on the complications of inguinal or subinguinal varicocelectomy techniques in the morbidly obese, data from inguinal hernia surgery reports a significantly higher infection rate in this population [14]. Given the increased skin to spermatic cord distance, increased tension on the spermatic cord is sometimes required to bring it high enough for adequate visualization during microscopic varicocelectomy, which may theoretically lead to worse outcomes, at least temporarily. Nevertheless, microscopic varicocelectomy has been shown to be equally effective in improving semen parameters and pregnancy rates in obese men as in normal-weight men [15].

However, embolization is not necessarily easier or safer in the morbidly obese. Vascular access in the morbidly obese is more difficult, even with ultrasound assistance [16]. Additionally, higher rates of vascular complications after catheterization procedures have been reported in morbidly obese patients [17].

Given the increased risk with all approaches to varicocele treatment in the morbidly obese patient, there is no optimal treatment approach when assessing the technical considerations of these procedures.

The Adolescent Patient

While the best approach to varicocele treatment in adolescents has not been established, laparoscopic varicocelectomy is more commonly performed than in the adult. A survey of pediatric urologists found that the most commonly used treatment approach to the adolescent varicocele was laparoscopic (38%) [18]. This is in contrast to the feasibility of microsurgical varicocelectomy in the adolescent population as modern series have shown similar, or lower, hydrocele and recurrence rates with subinguinal vs. laparoscopic approaches [19–22]. While the reason for a preference for the laparoscopic approach among pediatric urologists remains unclear, it may be due to differences in surgical training and comfort with these techniques.

Embolization is an accepted alternative in the adolescent patient, with several small series demonstrating success rates of >90% [23–25]. However, similar to adults, technical and anatomical issues lead to a failure to treat in these studies upward of 7%. Given these concerns, surgical therapy remains the preferred treatment of varicoceles in the adolescent patient; however, most pediatric urologists continue to prefer a laparoscopic approach.

Anesthetic Considerations

In contrast to surgical approaches, embolization is performed under sedation with local anesthesia [26]. While some surgeons may attempt microsurgical varicocelectomy under sedation or regional anesthesia, these authors highly recommend a general anesthetic. This is due to the need for fine microscopic dissection, as even small movements under the operating microscope are highly magnified.

Given the different anesthetic requirements between surgical and radiographic approaches, there may be a role for varicocele embolization in the patient who is unable to undergo a general anesthesia for varicocele treatment due to high risk or patient preference.

Comparison of Clinical Outcomes

The overall body of literature supports that treatment of varicoceles in general improves male fertility outcomes and scrotal pain. However, it is difficult to compare therapeutic outcomes among different techniques for varicocele treatment given the lack of randomized controlled trials.

Male Infertility

Historical meta-analyses of the available, but poor-quality studies led to historical controversy regarding the effectiveness of varicocele treatment for improving male-factor infertility. But high-quality data has since been produced, and

modern meta-analyses clearly demonstrate a benefit to varicocele repair for improving semen parameters and pregnancy rates [27–30]. The majority of this data focuses on outcomes from microsurgical varicocelectomy. A more recent randomized controlled trial comparing microsurgical varicocelectomy to nonintervention in infertile males with varicoceles and semen abnormalities demonstrated significant improvements in both semen parameters and natural pregnancy rates in the treatment arm (odds ratio of natural pregnancy 3.04) [31].

A recent meta-analysis performed exclusively to determine the best technique for fertility-focused varicocele treatment found that microsurgical varicocelectomy techniques had the highest overall spontaneous pregnancy rate at 41.97% compared to 33.2% in the embolization group [8]. A prospective, but non-randomized, study of men with infertility, semen abnormalities, and varicoceles undergoing either subinguinal microsurgical varicocelectomy or embolization demonstrated a similar improvement in sperm quality and pregnancy rates between the groups [32].

In summary, while varicocele treatment clearly improves male-factor infertility, the existing data does not clearly support one treatment approach over another in this regard.

Scrotal Pain

In a review of studies on the surgical techniques of varicocele for pain, microsurgical approaches have demonstrated the best overall success rate. A review of eight studies using microsurgical varicocelectomy demonstrated a mean complete pain resolution rate of 85% and a failure rate of 9% [33]. The same review examined six studies using non-microsurgical varicocelectomy techniques and demonstrated a mean complete pain resolution rate of 72% and failure rate of 10%. In three studies on laparoscopic varicocelectomy for pain, there was a mean complete pain resolution rate of 81% with a failure rate of 14%.

Some data exists supporting embolization as primary treatment of varicoceles for scrotal pain. One study demonstrated that 86.9% of patients had complete resolution of pain at 39 months follow-up after embolization [34]. However, failure rates with this approach tend to be higher than with microsurgical varicocelectomy [32].

As with the treatment of male-factor infertility, data supports the benefit of varicocele repair for scrotal pain, but it is difficult to determine a best technique given the lack of quality, comparative data.

Complications

Both surgical and radiographic approaches to varicocelectomy are generally considered low-risk procedures. However, each approach presents a unique complication profile, which should be considered when deciding on a treatment approach with each individual patient.

Surgical Treatment

Surgery involves general anesthesia to perform either a subinguinal, inguinal, or retroperitoneal incision or a laparoscopic approach to selectively ligate the internal spermatic vein while sparing the arteries and lymphatics. The complications inherent to surgery include recurrence, hydrocele, testicular pain, surgical site pain, testicular atrophy, bleeding, and infection. With the exception of varicocele recurrence and hydrocele, each complication generally occurs in 0–4% of cases depending on surgical approach [32, 35–37].

Despite the diversity of surgical approaches, all demonstrating improved clinical outcomes, only inguinal and subinguinal microsurgical varicocelectomy are associated with the lowest complication and recurrence rates [35, 37, 38]. Indeed, two recent meta-analyses support these findings by demonstrating a lower recurrence rate (1.05% vs. 2.6–14.97%), hydrocele rate (0.44% vs. 2.84–8.24%), and lower likelihood of overall complication (OR 0.05–0.07; 95% CI, 0.02–0.19) with microsurgical compared to retroperitoneal or laparoscopic approaches [8, 39]. Furthermore, the laparoscopic approach is uniquely associated with subcutaneous scrotal emphysema, inferior

epigastric artery injury, severe hemorrhage, and scrotal pain in up to 7% of patients [8].

While the routine use of an operative microscope during inguinal or subinguinal varicocelectomy has been challenged, it does provide superior visualization of the spermatic cord structures, mitigating recurrence and complication rates. Several series comparing microscopic assistance to loupe magnification or none at all have shown significantly lower recurrence (0% vs. 3–14.9%), hydrocele (0% vs. 2.9–5.9%), and testicular artery injury (0% vs. 9.2%) rates using the operative microscope [10–12]. As previously discussed, we recommend the routine use of an operative microscope and microvascular Doppler probe to allow for easier identification of the vascular anatomy.

Whether the inguinal or subinguinal approach is superior remains controversial. Anatomic data has demonstrated a higher number of spermatic vessels with the subinguinal approach due to distal vascular branching [40], which may explain some reports of shorter operative times with the inguinal approach [41]. However, others have shown no difference in operative times [42]. Inherent to the inguinal approach is a larger incision and division of the external oblique aponeurosis, which has been shown to result in increased postoperative pain [43].

Taken as a whole, when assessing complications, these data demonstrate that inguinal or subinguinal microsurgical varicocelectomy with intraoperative Doppler assistance is the gold standard of surgical therapy for varicocele repair.

Radiographic Treatment

In contrast to surgery, radiographic approaches are less invasive and can be routinely performed under local anesthesia or sedation. The predominant radiographic techniques include percutaneous embolization or sclerotherapy [44]. Given the different approach compared to surgery, radiographic treatments present a unique set of possible complications, including failure to treat (as previously discussed).

All radiographic approaches are associated with minor complications. The most common is post-procedural testicular pain or epididymitis persisting for up to 10 days (3–17%) [44–47]. Less commonly, an inguinal hematoma or contrast allergy may also occur [45, 48]. More controversial is the risk of hydrocele. Theoretically, hydrocele should not occur with radiographic approaches given isolated manipulation of the venous system, as shown in several series with zero reported hydroceles [23, 49]. However, one series reported hydroceles in 4.5% of cases [34].

Less common, but potentially higher risk complications have also been reported. Venous perforation with or without dissection into the renal vein or IVC can occur in up to 4% of cases, though often without clinical consequence [44, 48]. Also, endovascular coil migration to the right atrium and pulmonary arteries has been reported, potentially mitigated by proper selection of coil size [50, 51]. Lastly, ischemic colitis and bowel necrosis are rare but reported complications of the use of sclerotherapeutic agents [52, 53].

Theoretically, recurrences following radiographic approaches should be minimal given the operator's ability to identify all venous tributaries within and outside of the spermatic venous system [54]. Yet, recurrences still occur and have been reported in 0–24% of radiographic cases, although greater operator experience is associated with a lower risk of recurrence [8, 39, 44]. However, it is important to note the significantly lower range of reported recurrences with microscopic subinguinal or inguinal varicocelectomy of <2% [55].

In general, radiographic approaches offer a slightly less invasive treatment option that can be performed without general anesthesia but may result in rare, but serious complications and higher likelihood of recurrence than the surgical gold standard of microsurgical varicocelectomy.

Recurrent Varicocele

Regardless of the initial method used to correct a varicocele, recurrence rates are generally low. While the data evaluating treatment of varicocele recurrences are sparse, microsurgical varicocelectomy and percutaneous embolization are both viable options.

Surgical Treatment

There are three series to date evaluating the use of surgical intervention for recurrent varicoceles, but one employed a subinguinal approach without microscopic assistance and will not be discussed. The first includes 54 patients who initially underwent non-microscopic inguinal (74%), retroperitoneal high ligation (10%), microscopic inguinal (4%), or non-microscopic subinguinal (2%) approaches. Postoperative mean serum testosterone, testicular volume and median sperm concentration, percent motility, and total motile sperm count all demonstrated statistically significant improvement after undergoing repeat varicocelectomy via a microsurgical subinguinal approach with no reported recurrences [56]. The second series included 12 patients initially diagnosed with orchialgia, but the method of initial varicocelectomy is unknown. All patients underwent reoperation via a microsurgical subinguinal approach, and no recurrences were reported. Also, a favorable pain response was found in 91% of patients [57].

Radiographic Treatment

Percutaneous embolization offers a less invasive option for recurrent varicoceles as many may have initially undergone a surgical approach. Recurrent varicoceles are associated with increased collateral vasculature, which, in addition to a previously operated field, may make redo surgery technically more difficult [44, 58]. Indeed, up to 93% of recurrent varicoceles are due to incompetent gonadal veins, 66% of which are due to gonadal vein duplication, which is readily identifiable radiographically [54]. A recent series of 28 patients with recurrent left varicoceles after previously undergoing laparoscopic varicocelectomy (39%), retroperitoneal high ligation (25%), or inguinal varicocelectomy (25%) underwent percutaneous embolization, which was feasible in 93% of cases. Post-procedural success was determined by physical examination, revealing 80% of cases resolved, 16% improved, and 4% no change. In those with scrotal content pain, 83% showed resolution or improvement [59]. Other series utilizing either percutaneous sclerotherapy or embolization with post-procedural success evaluated by physical exam have shown similar results [54, 60, 61]. However, detection of recurrent varicocele by physical exam can be very subtle as thickening of the spermatic cord may persist despite resolution of the underlying vascular reflux.

In summary, recurrent varicoceles may be treated with either surgery or radiographic techniques, as insufficient comparable data is available to make a definitive conclusion. These authors advocate consideration of a radiographic approach in cases of varicoceles recurrence after surgery to mitigate the morbidity of a second operation. However, one could consider surgery in the context of a bilateral recurrence, recurrence after initial radiographic procedure, or if the redo surgery can be performed on a different segment of the spermatic cord than the initial operation.

Cost-effectiveness

When multiple therapeutic approaches exist to treat the same condition, one measure of comparison is cost. However, it is important to distinguish between the upfront cost and the overall cost-effectiveness of a treatment. Older studies reported that the cost per treatment was lower for embolization compared to the surgical approaches for varicocelectomy [62, 63]. However, these studies do not account for attempted embolizations that are aborted due to access failure, therapeutic ineffectiveness, or the treatment of recurrences. This is why a comparison of overall cost-effectiveness is more relevant.

A recent analysis demonstrated that microsurgical varicocele repair is more cost-effective than embolization in the treatment of male infertility [64]. Using data pooled from 33 studies, and taking into account the cost of treatment, the recurrence rate, the cost of retreatments, and the pregnancy rates, the authors performed a Markov decision analysis that demonstrated microsurgical varicocelectomy to be the most cost-effective primary treatment strategy for varicoceles. The

reported cost per pregnancy was about 25% less for microsurgical varicocelectomy than embolization.

Conclusion

While several options exist for varicocele treatment, the preferred primary approach is the microsurgical inguinal or subinguinal varicocelectomy. Since the different approaches have all been shown to be effective, but have not been readily studied prospectively, the preference for microsurgical varicocelectomy is largely based on the lower rate of complications and recurrences compared to other techniques. This approach also avoids the unique problem of failure to treat as with embolization procedures. However, there remains a role for embolization when proper surgical instrumentation is not available, anesthetic concerns exist, specific complications are of concern, or in the treatment of recurrent varicoceles.

Review Criteria

An extensive search of studies examining the surgical or radiographic treatment of varicoceles was performed using search engines such as PubMed, MEDLINE, and Google Scholar. Pertinent literature published within the past 30 years was evaluated. Literature describing the history of varicocele treatment published prior to the 30-year search period was also evaluated. In order to hone our search, the following keywords were used: "varicocele," "varicocelectomy," "varicocele repair," "varicocele surgery," "varicocele embolization," "varicocele sclerosis," "varicocele repair success," "varicocele surgery complications," and "varicocele embolization complications." Articles not published in English were not evaluated. For individual varicocele treatment modalities, meta-analyses, randomized controlled trials, and single-center or retrospective cohort series were evaluated. For treatment complications, meta-analyses, randomized controlled trials, single-center or retrospective cohort series, and review articles were evaluated. Data that were solely published in conference or meeting proceedings or websites were not included.

Multiple Choice Questions and Answers

1. Which of the following is a unique consideration specific to embolization compared to surgery?
   (a) Treatment of the obese patient
   (b) Treatment of the adolescent patient
   (c) **Failure to initially treat**
   (d) Type of anesthetic required
2. Which of the following is the only optical surgical instrument that has been shown to reduce postoperative complications following surgical varicocelectomy?
   (a) Magnifying loupes
   (b) **Operative microscope**
   (c) Laparoscopic camera
   (d) Robotic endoscopic camera
3. Which of the following complications is not associated with surgical varicocelectomy?
   (a) Incisional infection
   (b) Hydrocele
   (c) Varicocele recurrence
   (d) **Renal vein dissection**
4. Which of the following is the most commonly encountered complication following percutaneous radiographic treatment for varicocele?
   (a) Endovascular coil migration
   (b) Arterial perforation
   (c) **Testicular pain**
   (d) Inguinal hematoma
5. Which of the following surgical approaches to varicocelectomy has demonstrated superior complication rates in the pediatric and adolescent populations?
   (a) **Microscopic varicocelectomy**
   (b) Laparoscopic varicocelectomy

(c) Retroperitoneal high ligation varicocelectomy
(d) Percutaneous anterograde embolization

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47 Should Varicocele Be Managed Surgically or Radiographically? (Radiology)

Luke E. Sewall and Steven Janney Smith

Key Points

- The technique of percutaneous varicocele embolization has improved significantly since its inception in the late 1970s.
- Using current techniques, the technical and clinical success of varicocele embolization approaches 97%.
- Percutaneous varicocele embolization allows mapping and treatment of all feeding vessels leading to a varicocele on both sides.
- Close collaboration between urologists and interventional radiologists will lead to improved care for patients suffering from varicoceles.

Surgical varicocelectomy has been performed since 1949 [1]. Varicocele embolization is still relatively new, having been first performed in the 1970s [2]. There are several potential advantages of this nonsurgical technique including avoidance of general anesthesia, more rapid return to full activities, decreased risk of infection, and no risk of hydrocele. In addition, bilateral varicoceles can be treated from a single access. Nonsurgical treatment also avoids the risk of arterial and lymphatic injury. Therapeutic embolization involves blocking or redirecting blood flow by injecting coils, plugs, beads, liquid agents, and other materials with the intent of modifying or curing disease inside the body without surgery (Fig. 47.1). In the case of varicocele embolization, the intent is to accomplish a "ligation" of the refluxing vein leading to the varicocele by injecting agents under a venographic roadmap through a small catheter. Unless otherwise stated, this chapter will refer to retrograde embolization.

As improvements and innovation have changed surgery, varicocele embolization has become more technically successful over the

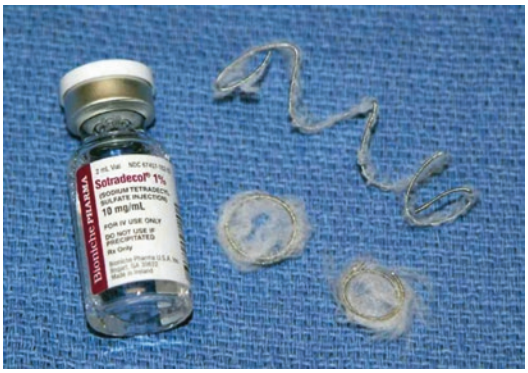

Fig. 47.1 Soft platinum coils with Dacron fibers and Sotradecol. These may be used in combination to produce varicocele occlusion

L. E. Sewall (✉) · S. J. Smith
Department of Interventional Radiology, Vascular and Interventional Professionals, Hinsdale, IL, USA

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years while improving its safety and efficacy. Still, the vast majority of patients are not offered embolization for a variety of reasons. Doubts about the success rate of embolization, based on decades old data, have been perpetuated in the urologic literature [3–6]. General concerns about danger from radiation exposure are commonly mentioned. Interventional radiologists may be seen as economic competitors or less skilled clinicians by urologists. Some interventional radiologists act as "proceduralists" and have no clinical infrastructure to handle problems, complications, or patient follow-up. The two specialties have not always meshed well in the instance of varicocele to the detriment of good patient care.

Evaluation for Embolization

Indications for embolization are identical to those for varicocele surgery. These are discussed elsewhere in this text. Relative contraindications include severe contrast allergy that cannot be safely premedicated or an active infection in the procedure area.

In our practice, all patients get a high-resolution ultrasound of the scrotum (Fig. 47.2) and an outpatient clinic consultation with the interventional radiologist. All embolization candidates are given an informed consent process including risks and benefits of all options for treatment.

In general, an embolization procedure typically takes about 30 min to perform. If the patient has variant anatomy or happens to be a prior surgical failure, the procedure can take up to an hour. The procedures are done under local anesthesia, and if the patient desires, light conscious sedation. Procedures are performed in a hospital, a surgicenter, or even a well-equipped office-based lab. After the procedure, patients are observed for 2–4 h in a standard recovery area prior to discharge to their home. They are told to call our clinical office if there are any problems and, in some cases, to expect some possible soreness for a few days. They may resume all normal activities in 24–48 h, except for heavy weight lifting, which they refrain from for one week.

Performing Varicocele Embolization

Varicocele embolization of the left or right internal spermatic vein (ISV) is usually performed with light conscious sedation (usually with intravenous Sublimaze and midazolam), fluoroscopic guidance, and sterile technique. Most interventionalists use real-time ultrasound guidance for accessing the vein. The most common approaches are the right inter-

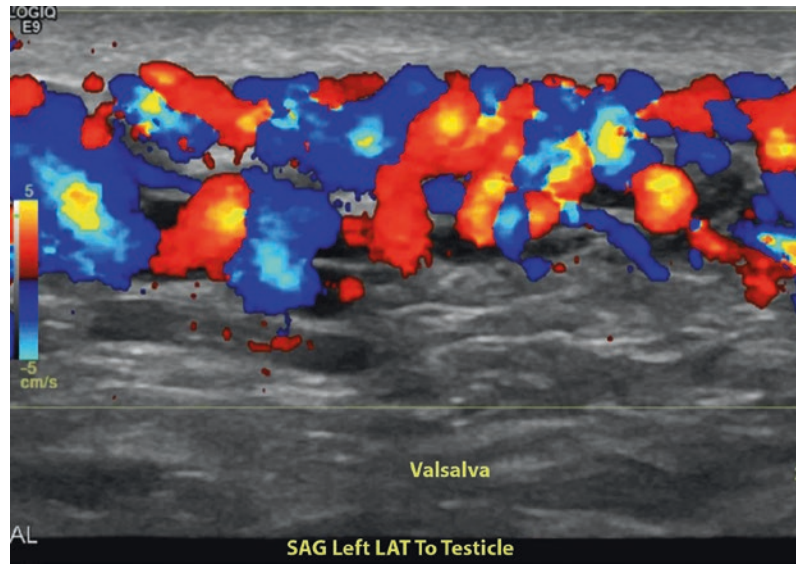

Fig. 47.2 A color-flow ultrasound image with Valsalva maneuver showing a prolonged surge of reflux in the pampiniform plexus indicating a left varicocele

nal jugular vein and the right common femoral vein, but arm veins or left-sided veins may be used as well. There is no reported difference in radiation exposure or success from any one approach; therefore, route of access is a matter of operator preference [7].

As the patient is put on the angiographic table, a lead apron is carefully placed to shield the gonads from direct radiation exposure. In early reports of radiation dose to the testis, thermoluminescent dosimeters showed the testicular dose to be about as low as received during typical diagnostic imaging procedures [8]. Now, using modern dose reduction equipment and virtual collimation and also by limiting digital subtraction runs, the absorbed dose is much lower [9]. Dangerous radiation exposure should never occur during varicocele embolization by an experienced operator using modern equipment [10].

One of the many benefits of percutaneous treatment is the ability to map all of the abnormal veins on both sides using contrast venography. To treat a left varicocele, an angled angiographic catheter is guided into the left renal vein, and a Valsalva maneuver is elicited during contrast injection. Reflux down the left ISV into a varicocele is the usual route of disease pathology (Fig. 47.3). A common technique involves occluding the ISV

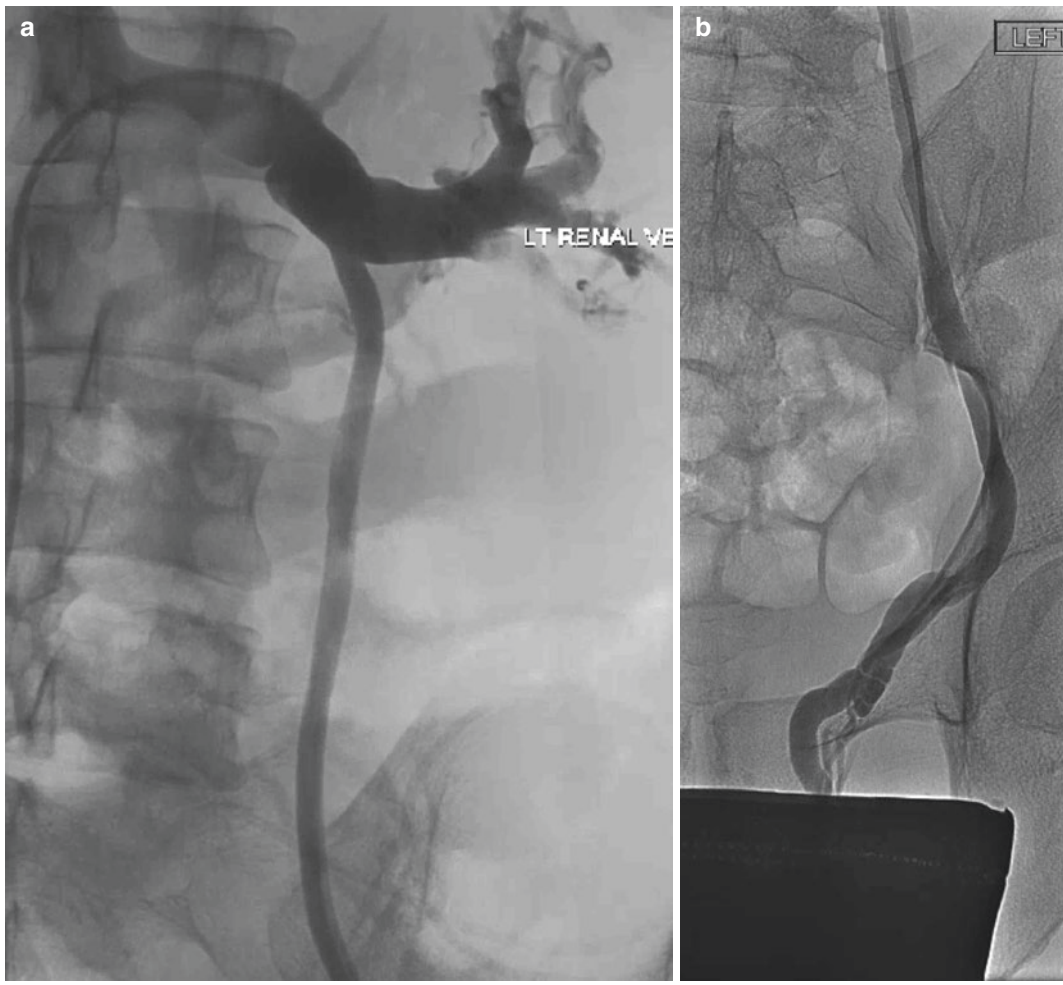

Fig. 47.3 (**a**) Standard left renal venogram during Valsalva showing left gonadal vein reflux. (**b**) Typical left gonadal venogram during Valsalva showing reflux all the way past the inguinal ligament into the scrotum. Note lead shield protecting gonads

just above the inguinal ligament with a few fibered coils and then injecting a sclerosant (we use 3% sodium tetradecyl sulfate solution) under direct fluoroscopy. The sclerosant is then followed by a few more coils leading to a "sandwich" technique (Fig. 47.4). The desired end point is sclerosis of all the small side branches (which, if untreated, can cause recurrence) and no reflux in the main branch. This technique prevents small natural collaterals from developing into new channels for reflux. Compression of the vein over the inguinal canal with a device or a lead glove during injection of sclerosant prevents reflux into the scrotum, which might cause phlebitis, but rarely does [11].

If the ultrasound detects a right varicocele, then the right ISV is also selected. This vessel usually originates off the IVC directly just below the right renal vein anterolaterally. Often, there is a valve at the junction. The embolization process is similar to that described for the left side. It can be difficult for the inexperienced interventionalist to catheterize the right ISV, and some reviews have described a lower success rate with right varicocele embolization [12]. This is a matter of experience, and with practice, the technical failure rate on the right should be very low (Fig. 47.5). Only 1% of varicoceles are right sided. There is also the phenomenon of "pseudo right varicocele" where a dilation of the right pampiniform plexus is pressurized by trans-scrotal collaterals from a refluxing left varicocele. These are cured by left varicocele embolization alone [2].

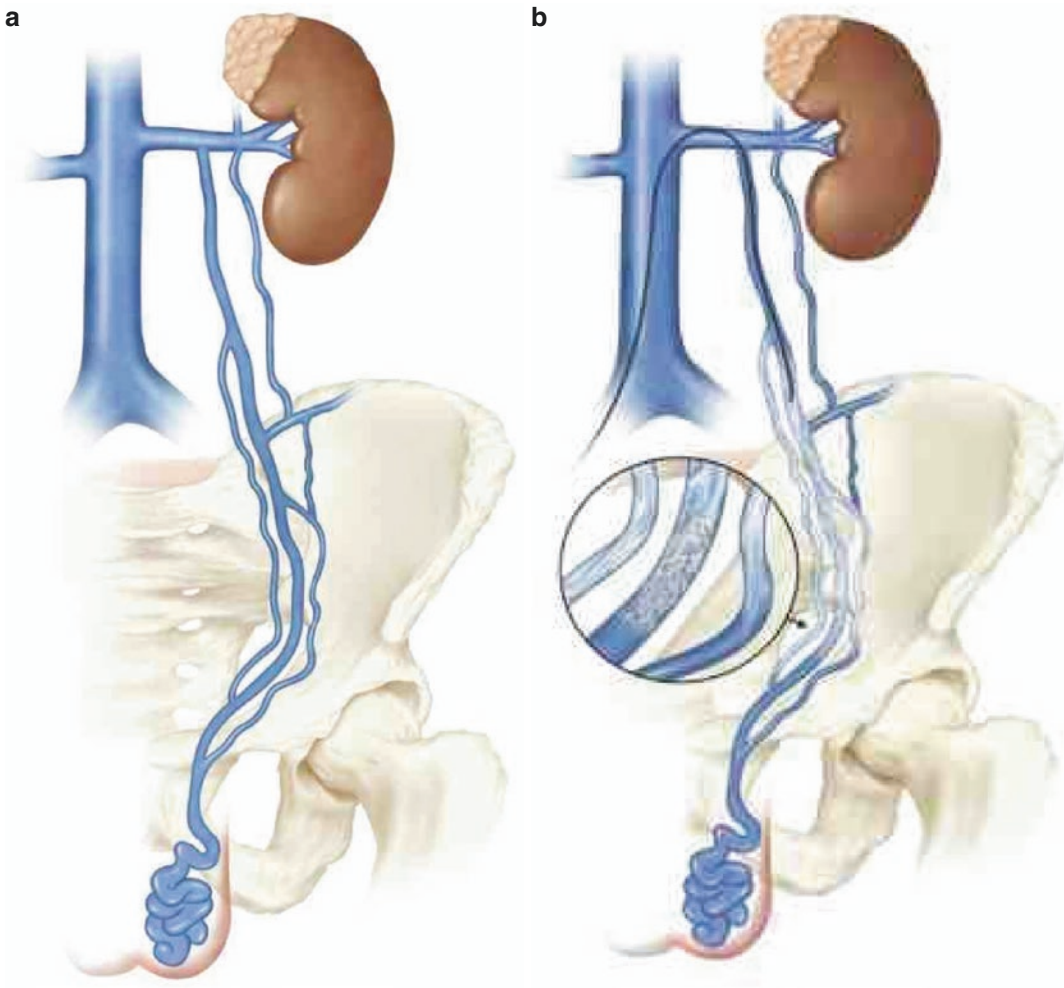

Fig. 47.4 (**a**) Left internal spermatic vein and branches. (**b**) Platinum coils and Sotradecol foam sclerotherapy illustrating the "sandwich" technique of varicocele embolization

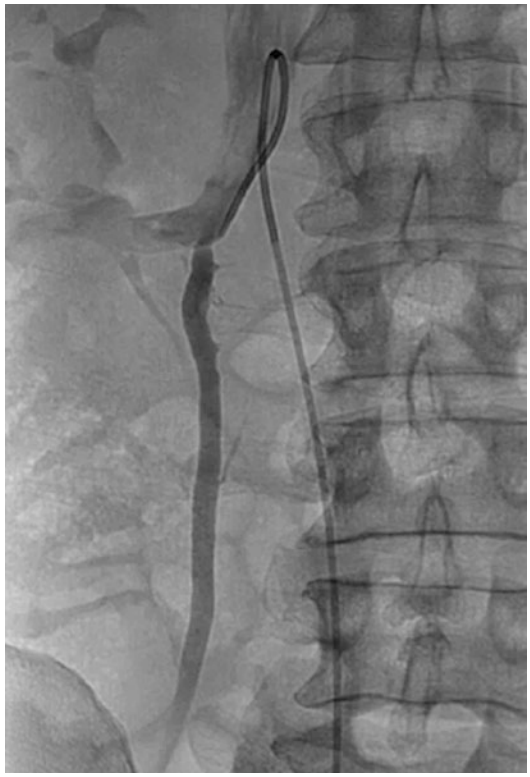

Fig. 47.5 The right ISV usually drains directly into the IVC but occasionally comes off the right renal vein

An aberrant varicocele may occur when reflux originates from another, sometimes hidden source rather than directly from the internal spermatic vein. While this was a major cause of technical failure of embolization 20 years ago when these alternate pathways were inaccessible to equipment available at the time, most can now be treated successfully. Commonly, renal hilar or capsular collaterals have a valveless connection and join the ISV diagonally in the retroperitoneum or pelvis (Fig. 47.6). Occasionally, an aberrant feeding vessel can be found using computed tomographic angiography with multiplanar reconstructions, which can spare a prolonged venographic search [13]. In fact, some of our more interesting cases are recurrence after surgical repair, where aberrant feeding vessels can be found and treated with embolization.

There are several different methods of varicocele embolization. Historically, occlusion balloons were used in the 1980s. Steel or tungsten coils were next used, but now platinum or nitinol coils may be used. We use platinum coils and Sotradecol foam in combination.

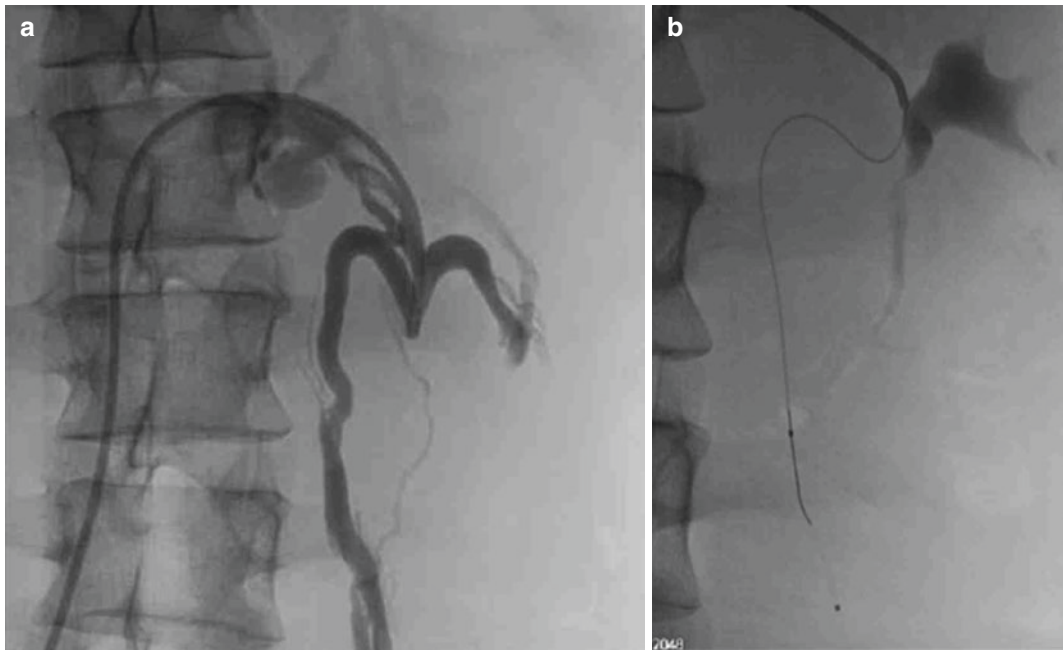

Fig. 47.6 (**a**) Left renal venogram shows an aberrant origin of a left varicocele from the renal hilum. (**b**) A 2-French microcatheter can be manipulated around several 180 degree curves to allow successful embolization. Newer tools like this allow technical success for embolization to rise to the high 90% plus range

Metallic coils may not be necessary for successful catheter-based treatment of varicocele. In Europe and Asia, retrograde injection of sclerosant alone is often used with a very high success and low recurrence rate [2]. In America, injectable glue is more expensive but is available [14, 15].

Complications of Varicocele Embolization

Serious complications of retrograde varicocele embolization have been reported, but rarely. Coils displaced to the heart or pulmonary arteries have been reported. In rare cases where they are symptomatic, they can be removed percutaneously with a snare technique as an outpatient [16].

Infection is vanishingly rare. Puncture site hematoma can occur and some soreness in the inguinal area. Minor perforation of small retroperitoneal veins can occur leading to prolongation or termination of the procedure. Scrotal phlebitis can rarely happen, if sclerosant travels down into the scrotum. Treatment is conservative and symptomatic with nonsteroidal anti-inflammatory drugs and a scrotal supporter.

Regarding complications of retrograde embolization vs surgery, the literature has few direct comparisons; but in 1994, DeWire et al. at Cleveland Clinic Urology performed a self-randomized trial comparing surgery and embolization. While there was no difference in efficacy between surgery and embolization, embolization resulted in much less time to complete recovery of full activity (24–48 h vs weeks) and far fewer complications. No embolization patient stayed overnight, and all infections occurred in the surgical group. One surgical patient had testicular infarction. Because of the fact that embolization was just as effective and had a much lower complication rate and time to recovery in this study, the authors concluded that embolization should be offered to all patients [17].

Since the complication rate of embolization is very low, success is high (see below), and return to full activities occurs more rapidly, this may be especially important to adolescent boys who often have very busy sports schedules. Embolization appears to have a near-zero incidence of hydrocele or testicular infarction [12]. Current complication rates for subinguinal microsurgery are lower than with other surgical approaches and are similar to embolization [12].

Antegrade Sclerotherapy

In 1994, Tauber, recognizing the benefit of sclerosing the microvenous collaterals in an effort to reduce surgical recurrence, described antegrade injection of sclerotherapy liquid after a subinguinal cutdown. This involved an incision and so was not as elegant as retrograde catheter embolization treatment. Others have attempted to follow his original idea, but results have not been promising, having a complication rate of 5% and a failure rate of 9.4% [18]. A modified percutaneous technique has been used by interventional radiologists using imaging guidance [19]. This can be a useful technique to treat a difficult recurrent varicocele, using real-time ultrasound guidance and limited fluoroscopy if necessary (Fig. 47.7). Blind injection of sclerosants into the ISV during antegrade sclerotherapy in the operating room should never be performed, however, since this has caused very serious complications in the urological literature, such as left colon infarction and paralysis [20–22].

Changes in Technology

In the early days of varicocele embolization (1980s and'90s), catheter and wire technology was quite primitive. Predominantly, only coils were used in the United States. This resulted in a reported technical failure rate for embolization as high as 24% during the 1980s. The recurrence rate for embolization at that time (with coils only) was variable but higher than reported for surgery. Both the higher technical failure rates and the variable recurrence rates were used to relegate the use of embolization to surgical recurrences mostly

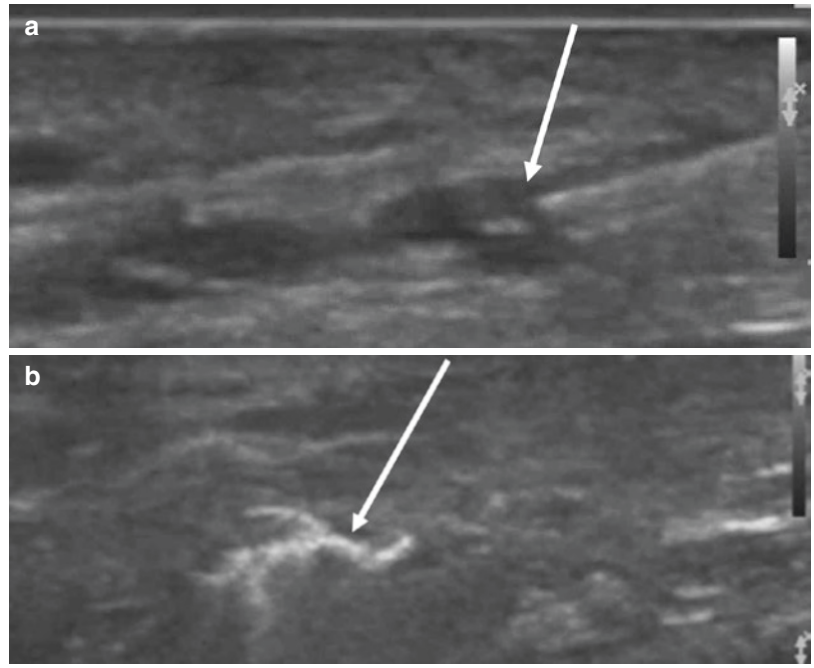

Fig. 47.7 (**a**) High-resolution ultrasound image showing a 21-gauge needle entering a collateral vein causing recurrent varicocele. The entry site is just below the external inguinal ring. (**b**) After injection of 1 cc of liquid 3% Sotradecol, the vein is in spasm with visible strands of sclerosant. The varicocele was completely resolved by this minor procedure

[21]. One commonly stated reason in the recent urologic literature that all patients should get surgery is that if subinguinal varicocelectomy has an almost 100% effectiveness, then all patients should have that surgery. In a counterargument, however, if a procedure is available that is greater than 95% effective, but is safer, with quicker recovery, and involves no incisions, one might suggest the number of patients needing surgery is only 4–5% rather than 100%. This of course is the subject of vociferous debate and in the literature has involved very selective use of statistics. For example, if a recent meta-analysis on success rate of embolization includes only data from the 1980s and 1990s, then the "success rate" will not reflect the improved efficacy of the procedure as it exists today [3, 6]. In Table 47.1, a grouping of varicocele embolization studies is stratified by decade. It can be seen that the *overall success* of varicocele embolization (factoring in both technical success and recurrence rate) has steadily risen by decades, from 58% to 59% to 93% to the current 97% in this decade. It is no longer accurate to state that there is a "20%" technical failure rate and so to dismiss embolization [22].

Pain Relief with Varicocele Embolization

In a review of 154 cases of coil embolization, Puch-Sanz found that pain relief dropped from a VAS 7 to average of VAS 0. Pain relief was noted nearly immediately after embolization, and at 39 months, 87% of patients had total relief of pain [21]. Embolization is effective at pain relief, and other studies have shown that it causes less pain than surgery [16, 44]. Varicocele embolization is as effective as surgery for pain relief and is less painful for patients to undergo.

Fertility and Pregnancy

Many studies comparing embolization and surgery have not used the gold standard of microsurgical repair, or have used old embolization studies, but no large recent randomized trial is available to compare the two treatments as they are performed today. Several recent reviews use the decade-old embolization data and recommend against embolization for fertility on that basis [6, 12, 45].

Table 47.1 Meta-analysis of varicocele embolization outcome by decade

Study	Year	Patient N	Technical success N	Recurrence N	Overall success N/Tot
Formanek et al. [23, 24]	1981	30	21	NR	–
Reidl et al. [25]	1981	64	51	6	45/64
Seyferth et al. [26]	1981	580	249	7	242/580
Berkman et al. [27]	1984	30	27	NR	–
Morag et al. [28]	1984	113	88	2	86/113
Porst et al. [29]	1984	279	234	7	227/279
Suarez et al. [30]	1986	18	9	NR	–
Total	**1980s**	1114	679	22/622	600/1036
Outcome percentage		100%	**61%**	**4%**	**58%**
Ferguson et al. [31]	1995	116	106	NR	–
Feneley et al. [32]	1997	154	125	NR	–
Abdoulmaaboud et al. [33]	1998	120	80	9	71
Total	**1990s**	390	311	9/120	71/120
Outcome percentage		100%	**80%**	**8%**	**59%**
Krause et al. [34]	2002	20	11	NR	–
Nabi et al. [35]	2004	102	98	2	96/102
Tanahatoe et al. [36]	2004	50	41	NR	–
Gandini et al. [37]	2008	280	272	10	262/280
Flacke et al. [38]	2008	223	212	NR	–
Reiner et al. [39]	2008	16	16	1	15/16
Bechara et al. [40]	2009	41	39	2	37/41
Total	**2000s**	732	689	15/425	410/439
Outcome percentage		100%	**94%**	**4%**	**93%**
Cassidy et al. [41]	2012	158	140	NR	–
Iaccarino and Venetucci [2]	2012	4000	≈3880	0	3880/4000
Urbano et al. [14]	2014	41	41	0	41/41
Zampieri et al. [42]	2015	184	172	11	161/172
Jargiello et al. [43]	2015	31	31	0	31/31
Total	**2010s**	4414	4264	11/4124	4113/4244
Outcome percentage		100%	**97%**	**0.3%**	**≈97%**

NR no report

A recent prospective comparison of subinguinal microsurgical varicocelectomy vs embolization by Bou Nasr et al. from 2017 in 76 patients found faster recovery and less postprocedure pain with embolization but equal outcomes for improvement of sperm quality, pregnancy rate, and overall satisfaction. Their conclusion was that the two procedures were equivalent, with slightly less morbidity for embolization. Infection and bleeding occurred only in the microsurgery group. Return to work occurred on average at 6.8 days for the surgery group, but embolization patients were back on average by 1.5 days, a significant advantage for embolization.

Embolization may also actually improve sperm quality and quantity *more rapidly* than microsurgical varicocelectomy. In a retrospective analysis of short-term improvement of sperm total motile count in 56 patients, between embolization and subinguinal microsurgical varicocelectomy, a Toronto group noted at 3 months a significant improvement in total motile count only in the embolization group. Four patients in the surgery group went from oligozoospermia to azoospermia. The difference in semen parameters was not significant by 6 months. They hypothesized that the stress of surgery or anesthesia was a factor. They suggested that embolization may be

superior therefore in cases where IVF is planned close to the time of varicocele repair [22].

Some recent reviews allow the general equivalence of surgery and embolization, stating that the method of varicocele repair should depend on the experience of operators available [2, 12]. In 2018, the availability of an experienced interventional radiologist in North America who can perform varicocele embolization is high. On the website varicoceles.com, more than 400 experienced interventional radiologists are listed. Despite that, with a nonsurgical safe and effective treatment option available, most patients are not offered a referral to an interventional radiologist for evaluation in the United States.

With contemporary data accurately showing the current state of safety and effectiveness of percutaneous treatment, true informed consent would require that varicocele patients would be evaluated fairly and offered the option of embolization if they are surgical candidates. In DeWire's paper, allowing patients to choose between the two procedures, 50% chose embolization. In another study of patients who had both procedures, all patients preferred embolization [16, 21]. Clearly, many patients were not being informed of the option to have nonsurgical cure of their varicocele, and there is evidence many would choose nonsurgical treatment. Interventional radiologists, in turn, must manage their patients and be involved in the clinical evaluation and follow-up, or their role will be nothing more than that of a glorified technician.

Patients will be the ultimate benefactors as both the surgical and nonsurgical treatment of varicoceles evolves and improves. Currently, percutaneous embolization offers a nonsurgical treatment of varicoceles that has been shown to provide equivalent success rates to contemporary surgery yet avoids many of the surgical risks. It is an outpatient procedure that allows rapid return to normal activities. By working together, urologists and interventional radiologists can provide the best options for our patients.

Review Criteria

Literature was reviewed involving the historical treatment of varicoceles dating up to the current state of the art treatments. Review included some surgical treatments but was centered on the endovascular treatment of varicoceles with a focus on retrograde embolization. Searches were conducted using MEDLINE, Google Scholar, and PubMed. Searches utilized the following keywords: "varicocele," "embolization," "sclerotherapy," "Sotradecol," "coils," "internal spermatic vein," "varicocelectomy," transcatheter therapy," "infertility."

Data from meetings, nonpublished sources, and websites was not utilized.

Multiple Choice Questions and Answers

1. Which of the following are common complications of varicocele embolization?
   (a) Testicular infarction
   (b) Hydrocele
   (c) More delayed improvement of semen analysis than surgery
   (d) All of the above
   (e) **None of the above**
2. Which are correct regarding varicocele embolization?
   (a) There is a longer recovery from the embolization procedure than for microsurgery.
   (b) Surgery is safer than embolization.
   (c) The recurrence rate for embolization is 20% or greater.
   (d) **In one study, 50% of patients chose embolization when allowed to select procedures.**
   (e) Embolization should not be performed on adolescent boys.

3. Radiation exposure during varicocele embolization:
   (a) Has caused several cases of sterility
   (b) Is a major risk of the procedure
   (c) Has gotten higher over the years
   (d) **Is decreased by shielding the gonads and limiting digital runs**
   (e) Should be emphasized to patients trying to decide between surgery and embolization
4. Total overall success of embolization for varicocele in this decade is closer to:
   (a) 67%
   (b) 71%
   (c) 85%
   (d) **95%**
5. Varicocele embolization has been performed successfully for:
   (a) 10 years
   (b) 20 years
   (c) 30 years
   (d) **40 years**

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Part VI

Varicocele Clinical Case Scenarios

48 Grade 1 Varicocele and Borderline/Normal Conventional Semen Analysis

Marco Grasso, Caterina Lania, and Angelica Anna Chiara Grasso

Key Points

Considering patients affected by low-grade varicocele and mild dyspermia, we can suggest that:

- Teenagers (<16 years old) should receive no treatment but be followed up after 2 years when sexual maturation should be complete.
- Men aged >16 and <30 years old with proved infertility should be treated.
- Men >30 years old with proved infertility and hypotrophic testicle on the side of varicocele should be treated.
- Men >30 years old with proved infertility and no hypotrophy of the testicle should not be treated.

Introduction

Varicocele is a condition characterized by the dilation of the spermatic veins forming the pampiniform plexus. According to the literature, varicocele affects approximately 40% of men with primary infertility and 80% of men with secondary infertility [1]. The first indication of this pathology and of the fact that it can result in testicular hypotrophy on the affected side dates back to Celsus, first century AD [2]. The first report of an improvement in sperm quality after surgical correction of varicocele was instead made by Barrel in 1885 and revised by Bennett in 1889 [3–4]. The incidence of varicocele in the general population is about 15%; it is therefore evident that not all carriers of varicocele are infertile [5]. The pathophysiology of varicocele-associated male infertility is not fully understood.

Certainly an important datum that we want to report, even if our work carried out on a very large number of patients is not yet published, is that in first-degree relatives of patients with high-grade varicoceles (those that are defined as grade 2/3 varicoceles with ultrasound diagnosis), the prevalence of venous pathologies, such as varicose veins of the lower limbs and hemorrhoids, is very high. This would suggest that the most important cause of varicocele is a venous dysplasia rather than other mechanical or biochemical mechanisms called in question in the past.

M. Grasso (✉)
Department of Urology, ASST MONZA, Ospedale San Gerardo, Monza, Italy

C. Lania
Department of Urology, IRCCS Ospedale San Raffaele, Milan, Italy

A. A. C. Grasso
Department of Surgery, Division of Urology, Ospedale Beata Vergine-Ente Ospedaliero Cantonale, Mendrisio, Ticino, Switzerland

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The objective of this chapter is to define the indication for surgery in patients who are suffering from a generically definable low-grade varicocele associated with modest alteration of the seminal fluid. In spite of differing opinions in recent years, the current trend in scientific literature has redefined the indication for the ligation of spermatic veins: it should be performed when the patient has a significant sperm alteration and a medium- to high-grade varicocele [6–7]. The gray area remains when there is a low-grade varicocele associated with a modest seminal alteration.

Definition of Clinical and Ultrasonographic Degrees

It is a general notion that the bearer of varicocele does not have a specific symptomatology even if in some articles the incidence of a sense of "weight" or the so-called bag of worms is reported to the affected testis. Symptoms are always defined as intermittent. It should be noted that intermittent testicular pain without an obvious pathology is one of the most frequent causes of urological examination in patients under 40 years. In our opinion, the presence of varicocele and pain should be considered as a coincidental finding, and it is therefore more appropriate to define varicocele as an asymptomatic pathology.

At this point, it becomes extremely important to investigate the pathology with a clear definition of clinical and sonographic degrees. Under the clinical profile substantially, we still refer to the first systematic classification made by Dubin [8], defining the varicocele in three grades.

For a better definition with the advent and the diffusion of the ultrasonographic instruments, using the Doppler pencil probe (Fig. 48.1), and then the color Doppler (Fig. 48.2), we arrived at an instrumental framework.

The Hirsh classification that has been used for many years [9] subdivides the fluximetric classification of varicocele into five degrees.

We have tried to make this classification clearer and more systematic, considering that the second and third degree are poorly definable and variable with respect to the experience, ability, and subjectivity of the examiner.

Fig. 48.1 Doppler pencil probe

Fig. 48.2 Eco-color Doppler probe

We also criticized the definition of the fifth grade because in our experience, spontaneous basal reflux is very rarely linked to non-variability during the Valsalva maneuver.

We have defined and described our classification [10], which includes three grades, simplifying and making the classification less subjective:

- First Grade: Identification of reflux during Valsalva maneuver
- Second Grade: Recording of spontaneous reflux during the patient's breaths
- Third Grade: Continuous spontaneous reflux, almost always increased, in our experience, by Valsalva maneuver

Often in case of first-degree varicocele in which the veins are not very ectatic, we can record the reflux with the "pencil" probe, of which we recommend the use in cases where basal eco-color Doppler can detect no significant venous ectasia [11].

We have always stressed that the examiner's ability and experience are fundamental. There should be other fixed parameters in the execution of the examination which are:

- A room with a temperature of not less than 20 degrees.
- The patient must be evaluated in orthostatic and supine position.

The evaluation of a patient affected by varicocele is therefore based mainly on three steps:

1. The clinical examination/objective examination
2. The ultrasound study performed with color Doppler or in alternative with velocimetry Doppler and pencil probe
3. Seminal examination

Management of Patients with Borderline Seminal Exam

For clinical examination and ultrasound studies, the methods of approach and evaluation are codified. Moreover, it is more complex to establish criteria for assessing normality concerning the semen analysis: it is in fact difficult to define in the evaluation of the patient's seminal exam the objective criteria that allow us to establish when a sperm test is normal or it is pathological, and in case it is pathological, if it may be due to the presence of a varicocele. Also for this reason, different and conflicting data on varicocele and fertility exist in literature [12–20].

A great effort has been made by the World Health Organization which has changed the parameters of a sperm test considered normal with respect to the previous classification dating back to 1999.

Eleven years later, in 2010, the parameters were modified, reviewing the data emerging from seminal assessments in healthy and fertile patients and in infertile patients and also using, for the identification of the actual fertilizing abilities, elements that come to us from spermatozoa recovered in the cervical mucus or from sperm successfully used for fertilization techniques such as ICSI.

This impressive study produced an attempt to standardization that substantially determined a reduction in the values of the so-called normal parameters both in terms of the number of spermatozoa, total and progressive motility, and morphology and vitality.

The World Health Organization has produced a substantial document [21] of guidelines for the implementation of a seminal examination, let's say ideal, to which we, readers who wish to evaluate the standards of their laboratories and implement the possibility of a diagnostic adequacy, refer. We have to note that, in our experience, few laboratories fully implement the recommendations produced by the WHO. We also note that it is true that the reading of a seminal examination carried out in a standardized way as suggested by the guidelines can reduce subjectivity of interpretation above all concerning the qualitative parameters, first of all the percentage of the normal forms.

This is a very important point because the indication to treat a low-grade or first-degree varicocele arises essentially when it is possible to define that the sperm of the infertile patient has parameters not in line with those established normal.

To clinically define an infertile patient, we can simplistically say that the patient has had a procreational relationship for more than 6 months. It is more difficult to give a subjective and not laboratory-dependent evaluation of its seminal parameters.

We consider it useful to address the description of the standard procedures made in the lines of WHO procedures. In particular, we wish to stress some points:

1. A semen analysis must be made strictly with an abstinence of not less than 2 days and not more than 7.
2. The patient should not have taken antibiotics within 6 weeks prior to the exam.
3. The emitted seminal fluid must be entirely collected.

A procedural error that in our experience is very frequent is not to give the importance it deserves to the loss of even a small part of the first ejaculate. If this happens, the exam must be repeated. We recall that the first fraction emitted is the prostatic fraction which is the richest in spermatozoa, while the last fractions are predominantly vesicular. For this reason, losing the first part influences the result more than the loss of the final portion.

It is also interesting to point out that the seminal fluid varies depending on the type of collection. In fact, the ejaculate produced by masturbation and collected in containers is often of lower quality than that recovered from condoms, without spermicides, used during a relationship. This is because the different excitation and the different pre-ejaculatory time affect the emptying of the seminal vesicles and the epididymal content.

In Table 48.1, we compare the parameters set by the WHO in 1999 and 2010, also highlighting those that in our experience we have arbitrarily defined parameters of "the gray zone" or uncertain interpretation and deserving individual therapeutic decision. It should be noted that the "lowest" seminal parameters are found in males under 30 years. Therefore, the modest alteration of a 25-year-old compared with a 40-year-old man has a different value [22].

We realize that this evaluation is subjective even if it arises from an experience of many years, but it is important to point out that on a parameter for a normal morphology of spermatozoa that falls from 30% in 1999 to 4% in 2010, there must be a margin for discussion. We underline that in our experience, it is imperative that the patient performs two seminal examinations at a distance of 30 days from each other before treatment decision for varicocele.

Table 48.1 Normal sperm value changes through the years

	Normal sperm value WHO 1999	Normal sperm value WHO 2010	Gray area
Volume	≥2 ml	≥1.5 ml	1/1.5
Concentration	20 million/ml	15 million/ml	10–15 million/ml
Sperm count	40 million	39 million	25–39 million
Total motility	≥50%	≥40%	25–40%
Progressive motility	≥25%	≥32%	20–32%
Morphology Normal forms	≥30%	≥4%	2–8%
Vitality	≥50%	≥58%	45–60%

We draw attention to the morphological parameters; in this case, the gray zone does not only include the values lower than the "normal value" but also the higher ones. In particular, if the patient is young, the limit of normality of 4% is acceptable at 40 years, certainly not at 20 years.

Although the exact mechanism how repair of varicocele would restore semen quality has not yet been satisfactorily explained, previous studies have reported an improvement in sperm quality postoperatively [23–24]. Steckel [25] stated that men with grade 3 varicoceles presented greater relative improvement in sperm count than men with grade 1 and 2 varicoceles. On the contrary, Braedel on a much larger cohort of patients (almost 700 vs. 86) [26] found less improvement in sperm count in men with grade 3 varicocele than men with lower-grade varicoceles.

At this point, we have to underline the importance of considering the testicular hypotrophy in varicocele as an indicator of decreased sperm counts.

As proved by Sigman on a cohort of more than 600 patients, testicular hypotrophy associated with unilateral varicoceles relates to worse semen

parameters than varicoceles without hypotrophy. These data support the practice of varicocele repair in adolescents and young adults to prevent or improve infertility [27].

The correct therapeutic approach to varicocele remains controversial, while in fact there is a widespread agreement on the opportunity to proceed with a ligation of the spermatic veins in cases where the patient is affected by a varicocele of second or third degree associated with a dyspermia or with a hypotrophy of the testicle [28]**.** The attitude to be held in the case in which the patient is affected by a low degree (or first degree) with a modest alteration of the seminal parameters remains the subject of debate [29].

We believe it is fundamental to make the evaluation both clinical and with ultrasound and seminal examination as objective as possible. Only in this way it will be possible to define a correct and absolutely individualized approach with particular regard to the patient's age [30–31].

We believe that it is appropriate to arrive at an increasingly simplified approach from the point of view of diagnosis and definition of the stage to facilitate a codification of the behaviors to be followed. We believe in particular that it would be advisable to reach a greater uniformity in the methods of execution of the ultrasound assessment with Doppler or eco-color Doppler and in particular a much more stringent coding for the execution and report of the seminal examinations. We recall that it is from the assessment of these two findings that the indication is given to the intervention, so borderline cases can be well interpreted only having a diagnostic procedures being performed correctly.

However, considering that the two tests used in the diagnosis of varicocele are unquestionably examiner-dependent, we believe that it will be very difficult to arrive at a net and universal definition of the criteria to be used for the surgical approach in borderline cases.

A meta-analysis showed that semen improvement is usually observed after surgical correction [32], and varicocelectomy can reverse sperm DNA damage [33]. The European Association of Urology guidelines suggest that men who have normal semen analysis and men with a subclinical varicocele should not be treated.

Considering all the reviewed literature and our own clinical experience of more than 40 years, we can therefore suggest to consider surgical treatment in infertile young men, especially if they have testicular hypotrophy and borderline semen analyses, because those are the patients who will probably benefit the most from the treatment.

Conclusions

The data retrieved from literature and our own experience suggest that young patients (<30 years old) with infertility should be surgically treated, because surgery has proved to improve the semen analyses and reverse sperm DNA damage and therefore lead to higher rates of spontaneous pregnancies.

Older patients should be treated only if they present with hypotrophic testicles because in those cases, varicocele probably plays a role in their infertility, whereas if there is no sign of hypotrophy, the varicocele is low grade and the sperm count is borderline, and the infertility is probably caused by a multivariety of factors which may not be corrected by surgery.

Review Criteria

A review of the literature was performed in May 2018 using PubMed, Cochrane Library, Scopus, and Web of Science databases. The search was conducted by typing the following terms: "low-grade varicocele," "normal semen analyses," and "varicocele treatment."

We have also reviewed the guidelines concerning the definition and treatment of low-grade varicocele with borderline/normal semen analyses, and we have summarized the retrieved material.

Multiple Choice Questions and Answers

1. According to the existing literature, which percentage of male infertility is caused by varicocele?
   (a) 5%
   (b) 25%
   (c) **50%**
   (d) 75%
2. According to the Hirsch classification of varicocele, how do we define a grade 3 varicocele?
   (a) Presence of minimum reflux at the beginning of the Valsalva maneuver
   (b) **Absence of baseline venous flow and reflux during the Valsalva maneuver**
   (c) Presence of basal spontaneous reflux steadily accentuated during the Valsalva maneuver
   (d) Presence of spontaneous reflux which increases slightly during the Valsalva maneuver
3. According to the 2010 WHO semen analyses guidelines, the patient who has to undergo the test must not have taken antibiotics for:
   (a) 3 months
   (b) **6 weeks**
   (c) 1 week
   (d) 3 days
4. According to the 2010 WHO semen analyses guidelines, the normal forms should be:
   (a) > 60%
   (b) > 30%
   (c) > 15%
   (d) **> 4%**
5. Considering a 35-year-old patient with proved infertility and varicocele, would you treat him with varicocelectomy?
   (a) Yes, always
   (b) **Only if he has an hypotrophic testicle**
   (c) Only if he doesn't have a hypotrophic testicle
   (d) No, never

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Grades 2/3 Varicocele and Normal Conventional Semen Analysis

49

Hussein Kandil and Rupin Shah

Key Points

- Men with normal semen parameters may have compromised sperm function, including elevated DNA fragmentation.
- Varicoceles may be associated with increased DNA fragmentation, and varicocele surgery can reduce DNA fragmentation.
- The current WHO norms for defining normal semen may not represent the optimal seminal parameters that give the best chance of fertility.
- There may be scope to improve the fertility potential of men with low-normal semen parameters by correction of a clinical varicocele.
- Some men may benefit from correction of a clinical varicocele despite having normal conventional semen parameters.

Introduction

Many men with varicocele will have normal semen analysis, no difficulty in impregnating their wives, and no pain or testicular atrophy. Surgery is not indicated for these men. After excluding surgery for testicular pain, current guidelines of the AUA, ASRM, and EUA recommend varicocele surgery only if there is infertility associated with an abnormality in the semen parameters, the varicoceles is clinically obvious, and the partner is potentially fertile [1].

In this chapter, we will seek to go beyond the scope of the guidelines and consider a controversial question: Could there be a role for ligation of clinical varicoceles in men with normal semen parameters (as per WHO reference values) when there has been no pregnancy despite their partners being normal?

Surgery for men with pain due to a varicocele, irrespective of semen parameters, is well established and will not be discussed here. Varicocele surgery to improve testosterone levels in a fertile man is also beyond the scope of this chapter.

There are two scenarios in which one may possibly consider varicocele surgery in a man with normal semen analysis whose partner is having difficulty conceiving:

H. Kandil
Department of Urology and Andrology, Fakih IVF Fertility Center, Abu Dhabi, United Arab Emirates

R. Shah (✉)
Department of Urology, Lilavati Hospital Research Centre, Mumbai, India
e-mail: rupin@vsnl.com

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1. Normal semen parameters, abnormal sperm function tests (especially high sperm DNA fragmentation, SDF), and clinical varicocele
2. Semen parameters at the lowest limit of normal range with clinical varicocele

Varicocele Surgery for Elevated SDF with Normal Semen Parameters

It is well recognized that conventional semen analysis has only a limited ability to predict male fertility, and many men with normal semen parameters may be unable to impregnate their wives [2]. Hence, a variety of sperm function tests have been developed [3]. Recently, especially after the widespread use of ICSI, the focus has been on measuring the quality of sperm DNA, by measuring sperm DNA fragmentation [4].

Given the variety of pathways through which varicoceles can affect fertility, it has been suggested that sperm function testing may give a better idea of the impact of a varicocele on a man's fertility [5]. An extension of this concept would lead to the speculation that there could be men whose fertility is compromised by a varicocele but whose conventional semen parameters are normal, and the only evidence of compromised fertility is increased reactive oxygen species (ROS) and consequently elevated DNA fragmentation index (DFI) [6, 7].

While there is a paucity of studies proving the value of varicocelectomy in men with normal conventional parameters and elevated DFI, there are many studies that validate the sub-concepts of this indication.

Incidence of High DFI in Men with Normal Semen Parameters

In an early study on the effect of DNA integrity of fertilization and embryo development, Sakkas et al. [8] found that sperm having high DFI may have normal morphology and motility. Summarizing results from recent studies on DNA fragmentation, Evgeni et al. [9] noted that while compromised semen parameters are often associated with high DFI, men with normal semen parameters also often have significantly elevated DFI.

In another study by Das M. et al. [10], which investigated the effect of paternal age on the sperm DNA, conventional seminal analysis failed to identify the spermatogenic disruption caused by aging in older men, though 17% of normozoospermic men over the age of 40 years had a high DFI of >30% (by sperm chromatin structure assay, SCSA).

The Danish first pregnancy planner study [11] assessed 215 couples and found that SCSA score correlated with fecundity. Fecundability declined as DFI increased and became small when aberrant cells were >40%. This was independent of conventional semen parameters.

Thus, there is considerable clinical data showing that many men with normal conventional semen analysis may still have compromised fertility due to raised DNA fragmentation.

Association of Varicocele with Elevated DFI

There are also a large number of studies showing that varicoceles lead to elevated DFI.

Smith et al. [12] studied DNA fragmentation in 55 men with varicoceles with normal and abnormal semen parameters and compared this with 25 normozoospermic donors. The DFI was tested using two methods – SCSA and TUNEL (terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labelling). ROS was also measured by chemoluminescence. They found very high levels of DFI in the men with varicocele and impaired semen parameters (SCSA, 35.5 +/− 9.0; TUNEL positive, 32.2 +/− 4.1) compared to the control group (SCSA, 7.1 +/− 0.9; TUNEL positive, 14.2 +/− 1.2). They also found high amount of DNA fragmentation in men with varicoceles and normal conventional semen parameters (DFI, 20.7 +/− 4.0; TUNEL positive, 26.1 +/− 3.2). ROS was also raised in men with varicoceles with normal and abnormal semen parameters.

Bertolla et al. [13] showed in an adolescent population with varicocele that sperm nuclear DNA fragmentation was overly increased within the varicocele group even when conventional seminal studies showed no evidence of abnormality. Taelbi et al. [14] confirmed increased levels of DNA abnormalities (measured by dye studies with aniline blue, toluidine blue, chromomycin A(3), and acridine orange) among infertility patients with varicocele compared to infertile men without varicocele and to fertile controls.

Thus, studies have shown that varicoceles tend to increase DNA damage significantly, and often these men may have normal conventional semen parameters.

Reduction in DFI Following Varicocele Ligation

The final question, then, would be whether correcting the varicocele in such men with normal conventional parameters but significantly elevated DFI would reduce the DNA damage and improve chances of conception [15].

The change in semen parameters and pregnancy rates after varicocele surgery in men with impaired semen parameters has been dealt with extensively in other chapters in this book. Here, we will look specifically for studies that assessed changes in SDF after varicocele surgery.

Zini et al. [16] looked at outcomes in 25 men after varicocele surgery. They found that post-op improvements in count and motility did not reach statistical significance, but DFI reduces significantly from18 ± 11% before surgery to 10 ± 5% at 4 months, and 7 ± 3% at 6 months, after surgery.

Similarly, in a study of 19 men who underwent varicocelectomy, Li et al. [17] found that while semen parameters improved postoperatively, they were still below control normal values. However, the DFI reduced from 28.4 ± 15.6% to 22.4 ± 12.9% which was similar to their control group.

In another group of 49 men with oligoasthenozoospermia and varicocele, DFI reduced from 35.2% to 30.2% after surgery [18], while Telli et al. [19] found a reduction in DFI from 34.5% to 28.2% in their group of 72 men.

In a recent meta-analysis of 12 studies on the effect of varicocele ligation on sperm DNA damage, Wang et al. [20] found that men with varicoceles had higher sperm DNA damage than controls, with a mean difference of 9.84% (95% CI 9.19 to 10.49), and that after surgery DFI reduced by 3.37% (95% CI −4.09 to −2.65). While this difference was statistically highly significant ($p < 0.00001$), the question is whether this amount of reduction in DFI would be clinically sufficient to impact chances of natural or IUI conception.

A similar doubt was expressed by Tiseo et al. [21] in their review of the outcomes of varicocele surgery. While concluding that surgery for palpable varicocele improved semen parameters, alleviated oxidative stress, reduced DNA damage, and could improve pregnancy rates, they commented that "given the low magnitude of the effect size in sperm DNA integrity, further research is needed to elucidate its clinical significance." Since SDF is the outcome of a synergistic action between external toxic factors and internal defects in spermiogenesis that predispose the sperm to damage [9, 22], it is possible that removal of a varicocele alone may not produce sufficient reduction in SDF.

In Zini and Dohle's systemic review of the relation between DNA fragmentation and varicocele [23], six of the seven studies reviewed showed that fertile men with varicocele had higher DFI compared to controls. While this data is quoted as evidence of the deleterious effect of varicoceles even in the presence of normal parameters and fertility, it also has another implication – that in many men, the elevated DFI caused by a varicocele may not impact fertility!

Thus, in men with a varicocele and elevated DFI but normal semen parameters, the recommendation for varicocele surgery can be made only with caution. The dots can be connected: men can have normal conventional parameters but elevated DFI; varicoceles do increase oxidative stress and increase DNA fragmentation; varicocele surgery often lowers DFI [24]. However, whether this will be clinically significant and will impact pregnancy rates can be asserted only after controlled studies.

In a SWOT analysis on sperm DNA fragmentation testing in clinical scenarios, Esteves et al. [25] suggested that for men with clinical varicocele, since 50% of those with normal semen will have elevated SDF, 70–90% will show reduction in SDF after surgery, and postoperative pregnancy rates are higher in those with lower SDF, it would be worthwhile considering surgery in men with grade 2 or 3 varicoceles, with normal semen, if DFI is high.

Thus, if a couple is interested in natural conception, and has been unsuccessful over a prolonged period of time despite normal semen parameters and no female abnormality, then correction of a clinical varicocele may be guardedly considered if DFI is significantly high.

If an infertile couple with normal conventional semen parameters but high SDF and a clinical varicocele is considering ICSI as the next step, then the role of varicocele surgery to lower DFI is even more controversial since elevated DFI has a limited impact on ICSI outcomes, with perhaps some impact on miscarriage and live birth rates [26].

Varicocele Surgery for Men with Semen Parameters at the Lower Limit of Normal

The latest WHO reference values for semen analysis are a statistical derivation from around 1900 semen samples of men who achieved fatherhood within 1 year of trying. The 5th percentile of the study population's values have been taken as the cutoff to define "normal" semen [27]. While this low reference value ensures that most men who may be able to initiate a pregnancy would be labelled normal, it probably labels many men with suboptimal semen as normal, since many men with subnormal semen can achieve a first pregnancy, thanks to a super-fertile young female partner who can compensate for the subfertile male. When one consider the 50th percentile of the study group, it becomes clear that the majority of fertile men have semen parameters which are far above the WHO norm (concentration, 73 million/ml vs 15 million/ml; motility, 61% vs 40%; normal morphology, 15% vs 4%). Thus, it is likely that men at the lower reference level, who have been labelled normal, are actually suboptimally fertile but will not be investigated and treated because they have been labelled as normal [28].

Also, the WHO reference values did not take into account the time to pregnancy. Would a man at the 50th percentile be "more fertile" and be able to achieve a pregnancy faster? If so, there would be merit in treating correctable factors even if semen is normal at the lower end of the reference range.

Zinaman et al. prospectively followed pregnancies in 210 couples over 12 menstrual cycles. Using LOESS local regression smoothing of the data, they found that the 12-cycle fertility rate began to drop when sperm count dropped below 30×10^6/ml, or a total count of 80×10^6, or critical morphology below 8% [29]. These parameters are well above the current WHO norms, thus suggesting that improving semen quality of men with low normal parameters may improve fertility.

Bonde et al. studied 430 couples who were trying to conceive over six menstrual cycles. Sixty-five percent of subjects with sperm concentration at or above 40×10^6/ml achieved pregnancy compared to only 51.2% for subjects with concentration landing below this level. Further, the probability of conception increased with increasing concentrations up to 40×10^6/ml [30].

Slama et al. [31] studied 942 pregnant couples. The time-to-pregnancy (TTP) was noted and correlated with sperm count, morphology, and MAI (multiple anomalies index). They found that TTP correlated with sperm concentration up to 55 × 10(6)/ml, and with morphologically normal sperm up to 39% (or 19% by strict criteria). An increase of 0.5 in MAI was associated with an adjusted rate ratio for the occurrence of a pregnancy of 0.68.

This finding was consistent with the results of a study by MacLeod et al., who studied part-

ners of females achieving pregnancy with no medical help, and showed that the median time to pregnancy was less for males with sperm concentration above 40 × 10^6/ml compared to those with concentration between 20 × 10∗^{6}/ml and 39 × 10∗^{6}/ml [32]. In another study by Eggert-Kruse et al., pregnancies were significantly increased, reaching three times more, when normal morphology (using strict criteria) was above 14% compared to those below (*34.3* versus *11.1%; P < 0.01*) [33].

Thus, there may be an increase in a man's fertility potential if his semen quality improves from the lower limit of normal. Since the majority of current reviews do suggest that varicocele surgery will improve semen parameters in many men [1], a guarded recommendation may be made: if a couple has been trying unsuccessfully for a natural pregnancy over a long period of time, the wife is perfectly normal, and the man has normal semen parameters but at the lower reference range, then correction of a clinical varicocele may improve his semen parameters and increase the probability of conception.

Conclusion

As per current guidelines, a man with a clinical varicocele but normal semen parameters does not need surgery. However, as has been argued in this chapter, there may be a cautious role for varicocele ligation in men with normal semen parameters in two situations:

(a) If the DFI is significantly elevated and there has been no natural pregnancy despite an adequate period of trying (1–2 years) or when there has been recurrent ICSI failure
(b) When the semen parameters are at the lower end of the normal range (as per WHO criteria), there has been no pregnancy over an adequate trying time (1–2 years), the wife is normal, and the couple wishes to continue trying for a natural pregnancy

Review Criteria

An extensive search for studies examining the relationship between varicocele, sperm function tests, and pregnancy outcomes was carried out using search engines PubMed and MEDLINE, with start date of January 2000 and end date of March 2018. Study identification and data extraction were based on the following keywords: "varicocele," "DNA fragmentation," "sperm function," "semen parameters," and "pregnancy rates." Selected papers prior to search dates were also reviewed when cross-referenced by current reviews. Only English language papers were considered.

Multiple Choice Questions and Answers

1. A 32-year-old gentleman presents with primary infertility of 5-year duration. He has several semen reports which are normal as per WHO reference values, and his wife's investigations are also normal. On physical examination, a grade 3 varicocele was detected on the left side. What would be the next step in the management?
   (a) Refer to assisted reproduction.
   (b) Immediate left microsurgical varicocelectomy
   (c) **Offer DNA fragmentation testing.**
   (d) Observation with timed intercourse

   Explanation: Since duration of infertility is 5 years, there is now a need for active management. As per current guidelines, varicocele ligation is not indicated since conventional semen parameters are normal. ART would be the next step. However, as discussed in this chapter, there is a case for checking DFI; and if that is very high, despite the normal conventional parameters, then varicocele ligation could be considered before advising ART.
2. A 34-year-old gentleman presents with secondary infertility. He has been married for

8 years and has a 6-year-old daughter who was conceived naturally. They have been trying, unsuccessfully, for a pregnancy since the last 4 years. His latest semen parameters are within normal WHO parameters (2.5 ml, 18 million/ml, 40% progressive motility). However, compared to previous reports, there has been a gradual decline. Physical examination reveals a grade 3 and grade 2 varicocele on his left and right sides, respectively, with no other anomalies. His wife is normal. They have failed two consecutive intrauterine inseminations. What is the next step?

(a) Do more semen tests for better diagnosis, since semen parameters fluctuate a lot.
(b) Give them more time to try naturally since there has been one natural pregnancy and parameters are normal.
(c) Proceed to IVF/ICSI.
(d) **Varicocele ligation**

Explanation: Though semen parameters are normal, they are at the lower range of normal. As discussed in this chapter, this can be associated with reduced fertility. Also, the gradual decline in semen parameters suggests that the varicocele may be compromising testicular function. Hence, correcting the varicocele, despite "normal" parameters, may help the couple achieve natural pregnancy without the need for IVF.

3. A young couple, both 25 years old, present with primary infertility of 2-year duration. His seminal parameters are within the normal range on repeated testing. Physical examination reveals a grade 3 varicocele on the left side, and ultrasound examination also shows reflux on the right side. He had previously undergone DNA fragmentation testing by the TUNEL method which showed a high DFI of 40%. What is the best advice to give to this patient?

(a) Refer for assisted reproduction.
(b) **Bilateral varicocele ligation and then repeat DNA fragmentation testing after 3–6 months**
(c) Give them more time for natural conception.
(d) Give antioxidants

Explanation: The decision here is more controversial. The couple is young and trying time is short; hence, giving more time for natural conception is justified. However, DFI is very high and is likely to impair natural fertility and compromise ART outcome. Antioxidants can be given but have not been proven to reduce DFI. As discussed in this chapter, there is considerable evidence that varicocele ligation can reduce DFI and hence would be preferred if the couple wishes to enhance their chances of natural conception, before resorting to ART.

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50 Clinical Varicocele and Severely Abnormal Semen Analysis in a Couple Considering ART Whose Female Partner Is Over 36 Years Old

Luriel I. Smith-Harrison and Jay I. Sandlow

Key Points

- Couples where the female partner is of advanced maternal age warrant special counseling and management.
- Treatment of varicoceles may limit progression of testicular dysfunction.
- Treatment of varicoceles may allow for less invasive options and/or decreased cost.
- Even men with severe oligoasthenozoospermia may benefit from varicocele treatment.
- There is no right answer. The key is patient-centered care that allows for informed decision-making.

Introduction

Review of Incidence/Pathophysiology

A varicocele is defined as the abnormal dilation of the pampiniform plexus, a network of veins that drain the testes. Typically, varicoceles have been reported to be present in 15% of the general male population [1]. This widely held statistic is supported by a recent study of six European countries where 15.7% of healthy young men were diagnosed with a varicocele [2]. While relatively common in the general population, the prevalence of varicoceles increases for those men for whom fertility is a concern. Nearly 40% of men with primary infertility will be diagnosed with varicoceles, while approximately 80% of men with secondary infertility have varicoceles [3, 4].

While the etiology and pathophysiology of a varicocele are unproven, it is thought that poor venous drainage of the testis is the underlying cause. This insufficient drainage is thought to have three primary etiologies: [1] a more acute angle of insertion of the left spermatic vein into the left renal vein, when compared to the right, [2] retrograde venous flow due to insufficient or absent venous valves, and [3] decreased venous outflow due to the "nutcracker effect" or compression of the left renal vein by the aorta and superior mesenteric artery [5]. Testicular hypoxia, increased testicular temperature, elevated levels of reactive oxygen species, and buildup of metabolites and toxins have all been postulated as having a potential role in testicular insult and dysfunction [6].

Multiple studies have demonstrated the deleterious effects of varicoceles on semen parameters. Varicoceles are known to effect sperm count and motility, two of the most important factors for fertility. In the aforementioned European study involving over 7000 young men, sperm concentration, total

L. I. Smith-Harrison · J. I. Sandlow (✉)
Department of Urology, Medical College of Wisconsin, Milwaukee, WI, USA
e-mail: jsandlow@mcw.edu

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sperm count, progressive motility, and morphology were all significantly decreased in men with varicoceles. Of note, this difference was more pronounced with higher grades [2]. A study by Dieamant et al. also demonstrated increased rates of DNA fragmentation, abnormal chromatin packaging, and abnormal sperm apoptosis [7]. There is evidence that varicoceles may also lead to a progressive decline in testicular function [4]. The potential for progression of dysfunction is important when counseling patients and their partners.

Review of Advanced Maternal Age and Impact on Fertility

One of the prime determinants of fertility is female age. Studies suggest that women reach a peak of fecundity in their mid-20s to early 30s. Following that, a steady decline in pregnancy rates was appreciated with a 40-year-old woman having about half the chance of conception as her 30-year-old counterpart [8, 9]. Similar rates were reported by Menken et al., which shows historical data describing fertility and its relationship with age [10]. Age-related infertility in women is multifactorial. The most important factor is a progressive decline in ovarian function over time, including poorer quality and fewer oocytes. Faddy et al. demonstrated that the rate of ovarian function decline increases significantly after the age of 35 [11, 12]. There is also evidence of increased rates of chromosomal abnormalities or aneuploidy in women of advanced maternal age (AMA), which has been defined as pregnancy at or over the age of 35. This can be associated with failure of the embryo to progress through development [13].

Despite the gradual decrease in fecundity as women age, the medical community has pioneered interventions that can aid in achieving pregnancy utilizing assisted reproductive technology (ART). These techniques include intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI). In an effort to promote responsible use of resources, the American College of Obstetricians and Gynecologists (ACOG) and the American Society for Reproductive Medicine (ASRM) have issued opinion statements on management of this patient population.

When women are over the age of 35, ACOG and ASRM recommend expedited evaluation and treatment after just 6 months of unsuccessful attempts at pregnancy. At the age of 40, immediate evaluation and treatment is warranted [14]. The emphasis here is on time, with concern for continued loss of potential fertility. This same focus on timely intervention should also be utilized when counseling the male partner, keeping in mind that the male is only half of the equation.

Intervention for Varicoceles and Outcomes

Following guidelines set forth by the ASRM and the American Urological Association (AUA), correction of a varicocele is warranted when [1] the varicocele is palpable on physical exam of the scrotum, [2] infertility has been established and documented, [3] the female partner has normal fertility or a potentially treatable cause, and [4] abnormal semen parameters or semen function tests are found [15]. Treatment options include percutaneous embolization or surgical intervention. Surgical approaches are various and include laparoscopic, macroscopic inguinal ligation and microscopic inguinal or subinguinal ligation, although microscopic approach has been shown to have the highest success rate with the least number of complications [16, 17].

Multiple studies have shown improvement in semen parameters following treatment of a clinical varicocele. A randomized controlled trial by Abdel-Meguid et al. demonstrated that treatment of a varicocele led to improvements in both sperm count and motility [18]. This improvement was even seen in men with severe oligoasthenozoospermia [19]. Treatment has also been shown to decrease the levels of DNA fragmentation [20]. Despite the fact that there is good evidence to support varicocelectomy for improving semen parameters, there is still significant debate over varicocele treatment in the setting of expected ART use.

Treatment of Varicoceles and Effects on ART

When managing a couple with fertility concerns, it is vital that each partner's treatment goals take into

consideration the equally important goals of their partner. This is particularly true when deciding whether to pursue treatment of a varicocele prior to any attempts at ART. We will now review the relevant data on treatment of varicoceles and any effects it may have on ART. Though the data is sparse, we will also examine how this may affect a specific population, those men with severely abnormal semen parameters.

Effect of Varix Ligation on IUI

Intrauterine insemination (IUI) is the process by which semen is placed directly into the uterus following isolation of motile sperm. This process bypasses the vagina and cervix, allowing for a higher concentration of intrauterine, motile sperm. Data suggests that IUI is beneficial in couples with mildly abnormal semen parameters [21, 22]. While treatment of varicoceles is known to improve semen parameters, there is conflicting data on its effect on IUI. Daitch et al. reported that pregnancies and live birth rates were statistically higher with treated patients. Interestingly, there were no differences in the post-wash total motile sperm counts between groups [23]. In contrast, a retrospective analysis by Marmar et al. did not find a statistical improvement in men that were treated [24]. A more recent retrospective analysis by Boman et al. demonstrated that while rates of successful IUI attempts were higher in treated couples, this only trended toward significance [25]. The lack of convincing data highlights the need for further study in this area. Regardless, varicocele treatment prior to IUI is not unreasonable and should be offered to patients in accordance with established guidelines.

Effect of Varix Ligation on IVF/ICSI

In vitro fertilization (IVF), with or without intracytoplasmic sperm injection (ICSI), provides another option for ART. A meta-analysis by Kirby et al. showed increased pregnancy and live birth rates when treating a varicocele prior to IVF/ICSI (OR = 1.699) [26]. This meta-analysis included a study by Haydardedeoglu that specifically examined men with non-obstructive azoospermia. For men that were treated, sperm retrieval rates were significantly higher. They also experienced significantly higher pregnancy and live birth rates following IVF/ICSI (74.2% vs. 52.33% and 64.5% vs. 41.5%, respectively) [27]. A study by Guo et al. suggests that there may even be a role for varicocele treatment for severely oligoasthenozoospermic males after IVF/ICSI failure. Following treatment, upward of 60% of patients were able to conceive, with 22% of pregnancies naturally conceived. The remainder were conceived via IVF/ICSI [28]. This body of work would suggest that treatment of a varicocele prior to IVF may be reasonable. Again, the key to management is patient-oriented discussion and management.

Discussion/Debate

With a dearth of supporting data, there is considerable debate over the utility of performing a varicocelectomy in the setting of advanced maternal age and potential ART. When guiding a patient through the process, presentation of all risks and benefits is of paramount importance. Below we will present potential arguments for and against intervention.

Arguments for Intervention

Possibility of improving semen parameters to allow less invasive ART (or none at all) – One of the most compelling arguments for intervention is the potential to improve semen parameters enough to allow for less invasive ART. A study by Samplaski et al. showed that 58% of men that would have proceeded with IVF had substantial improvement in semen parameters that allowed for conception by natural means or IUI. The most significant improvements were seen in men with total motile sperm counts (TMSC) less than five million [29]. A study by Tanahatoe et al. demonstrated that 54% of men treated with varicocele embolization had significant improvement in semen parameters and conversion to less-invasive ART [30].

Prevent progressive decline of fertility and limiting ART options – There is significant evi-

dence that the deleterious effect of a varicocele is progressive. The high incidence of varicoceles in secondary infertility would support this [4]. A prospective study by Chen et al. examined this progressive deterioration. For men with known semen abnormalities, 87.5% will experience a further decline in parameters. Of the 28 men with deteriorating semen parameters, 10 experienced the decline within the first year of follow-up. For those men with normal semen parameters, 22% of men still experienced a decline [31]. Tanahatoe et al. demonstrated that with intervention only 10% of men would deteriorate to an extent requiring more invasive ART. For those that did not intervene, 44% experienced significant deterioration [30]. These studies may support treatment as a protective measure.

Cost – For a significant number of couples, management of infertility comes at significant cost and financial burden. Penson et al. examined four different treatment algorithms and found that immediate IVF was more costly per delivery and less effective than varicocelectomy coupled with IUI or IVF [32]. A prospective study by Dubin et al. reported that varicocelectomy coupled with IUI was more cost-effective than immediate IVF. This study specifically addressed men with severe oligozoospermia [19].

Arguments Against Intervention

Advanced maternal age as an overriding factor – Age is seen as one of the largest barriers to conception, especially when the female partner is over the age of 35 [8]. With age being the primary determinant of success, it could be argued that the risks of intervention outweigh any minimal improvement in pregnancy rates. Further quantifying the likelihood of conception by determining ovarian response can give insight into likelihood of success [33]. For those older women who already have poor ovarian response, treatment of a varicocele may be of little use.

Time lost while waiting for improvement in semen parameters – Improvements in semen parameters following varicocelectomy are not immediate. With a complete sperm cycle taking about 74 days, one would not expect to appreciate any improvements prior. Prior studies would suggest improvement in semen parameters occurs between 3 and 6 months [34]. For a woman of AMA, a delay in care of 6 months could be detrimental and decrease the odds of conception.

Overview and Summary

Any decision to proceed with an elective procedure should be based on patient-centered care where the goals of the patient or couple are clear. When debating the utility of varicocele treatment in a couple with multiple concerns (advanced maternal age and poor semen parameters), there are several key factors that should be addressed: interventional risks, expected outcome of the procedure, impact on pregnancy rates, timing, and cost. Only through an individualized approach will a provider be able to counsel a couple appropriately. As is the case with much of infertility, there is no right answer.

Review Criteria

An extensive search of studies examining the relationship between varicocele, assisted reproductive technology, and advanced maternal age was performed using search engines such as ScienceDirect, PubMed, Ovid, Google Scholar, and MEDLINE. The start and end date for these searches were April 2000 and April 2018, respectively. The overall strategy for study identification and data extraction was based on the following keywords: "varicocele," "assisted reproductive technology," "in vitro fertilization," "intracytoplasmic sperm injection," "advanced maternal age," "infertility," "subfertility," and "semen parameters." Articles published in languages other than English were not considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included. Websites and book chapter citations provide conceptual content only.

Multiple Choice Questions and Answers

1. Advanced maternal age begins at:
   (a) 25 years
   (b) 30 years
   (c) **35 years – correct**
   (d) 40 years
   (e) 45 years
2. At the age of 35, the ACOG recommends workup:
   (a) Immediately upon presentation
   (b) **After 6 months of failed attempts at conception – correct**
   (c) After 12 months of failed attempts at conception
   (d) Is no longer warranted
   (e) Only for the female
3. Which of these approaches has been shown to have the highest rate of success with the lowest rate of complications?
   (a) Laparoscopic varix ligation
   (b) Macroscopic inguinal varix ligation
   (c) Macroscopic retroperitoneal varix ligation
   (d) **Microscopic subinguinal varix ligation – correct**
   (e) Percutaneous radiographic ablation of varicocele
4. All of the statements below would argue for intervention *except*:
   (a) Potential to improve semen parameters and allow for less invasive ART
   (b) Decreased financial burden
   (c) Improved DNA fragmentation rates
   (d) **Allow for immediate improvement in semen parameters – correct**
   (e) Prevent progressive decline in semen parameters
5. When compared to a 30-year-old counterpart, a 40-year-old woman has:
   (a) 10% the chance of conception
   (b) 33% the chance of conception
   (c) **50% the chance of conception – correct**
   (d) 75% the chance of conception
   (e) 90% the chance of conception

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Asymptomatic Male with Grade 3 Left Varicocele and Two Children with Low Testosterone Levels Desiring Vasectomy

Rodrigo Uliano Moser Da Silva
and Daniel Suslik Zylbersztejn

Key Points

- Vasectomy appears as one of the main contraceptive options and the only safe surgical technique until this moment for male contraception. It is one of the most effective methods of contraception, with a 0.05% risk of failure.
- The adverse effects of the hyperthermia-hypoxia-oxidative stress cascade generated by clinical varicocele on male spermatogenesis and infertility are already well known, but there is much less knowledge about the effects on testosterone production.
- Clinical varicocele seems to be an androgen deficiency-independent factor, and its microsurgical correction may raise previously low levels, but with low efficacy in patients with testosterone levels considered normal for age.
- Instead of raising testosterone levels through the use of exogenous androgens, detecting the cause of hypogonadism and correcting the primary cause can bring much more health benefits, even in men whose future fertility is no longer a concern.
- Performing a surgical technique that preserves the different arteries to the testis (testicular, cremasteric, and deferential) can generate an increase in testosterone levels, able to prevent or delay the clinical onset of hypogonadism in a patient with low testosterone.

Introduction

Sexual and reproductive health care is considered an intervention tool for improving well-being. Contraception is a relevant topic because of its widespread and prolonged use throughout the world. The contraceptive method chosen has to be effective and compatible with the clinical, psychological, social, and cultural profile of the individual [1]. When addressing the topic of family planning, vasectomy appears as one of the main contraceptive options for couples and the safest surgical technique available today for men. It is one of the most effective methods of contraception, with a 0.05% risk of failure when performed by experienced surgeons [2]. It was estimated that approximately 527,476

R. U. M. Da Silva
Department of Urology, Hospital de Clínicas De Porto Alegre, Porto Alegre, Rio Grande Do Sul, Brazil

D. S. Zylbersztejn (✉)
Fleury Group, São Paulo, SP, Brazil
e-mail: dr.daniel@dszmedicinareprodutiva.com.br

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vasectomies were performed in the United States in 2015 [3]. Although vasectomy poses no health risks, it is not free of complications, such as infection, hematoma [2], as well as chronic testicular pain, also known as chronic orchialgia, which affects 1–2% of patients [4].

Varicocele is the most important and frequent disease related to male infertility, affecting 15–25% of male population. Varicocele represents 40% of primary and 80% of secondary causes of male factor infertility [5]. More recently, researches have shown that clinical varicose veins may be related to decreasing testosterone production and the onset of male hypogonadism [6, 7].

Surgeons should inform patients of possible postoperative clinical consequences, in addition to the inherent risks of vasectomy, when vasectomy is associated with asymptomatic grade 2 or 3 varicocele and low total testosterone level. Thus, the physician should be able to discuss the surgical treatment best suited to the patient's clinical needs.

To better understand the consequences of vasectomy in patients with clinical varicocele and low testosterone level, this chapter will cover (i) testicular arterial anatomy; (ii) the relationship between clinical varicocele, its surgical repair, and hormone balance in men; and (iii) a differentiated surgical strategy for patients with these clinical characteristics, aiming at obtaining a safe contraceptive method, increasing the chances of improving total testosterone level, and contributing to the delay or even resolution of clinical hypogonadism.

Testicular Arterial Anatomy

The arterial supply of the testis comes from three sources: the internal spermatic artery (testicular artery), deferential artery (vasal artery), and external spermatic artery (cremasteric artery). The testicular artery originates from the aorta just below the renal artery. It joins the spermatic cord just above the internal inguinal ring and follows a path adjacent to the pampiniform venous plexus to the mediastinum testis, establishing a mechanism for maintaining testicular temperature by countercurrent exchange. Near the mediastinum testis, the internal spermatic artery is highly convoluted and bifurcates before entering the testis. Extensive interconnections between the deferential artery and internal spermatic artery may allow maintenance of testicular viability even after internal spermatic artery ligation. However, this is less likely to occur when it is ligated in the subinguinal region, because the testicular artery becomes a final artery without anastomoses. The deferential artery arises from either the superior or inferior vesical artery and irrigates the vas deferens and tail of the epididymis. The external spermatic (cremasteric) artery originates from the inferior epigastric artery at the internal inguinal ring, where the spermatic cord enters [8].

Varicocele, Its Surgical Repair, and Hormone Balance in Men

The adverse effects of hyperthermia-hypoxia-oxidative stress induced by clinical varicocele on spermatogenesis and male infertility are already well known, although there is still controversy over some of the pathophysiological mechanisms involved. The quantitative and qualitative effects of varicocele surgery on spermatogenesis have also been extensively studied, especially from the middle of the last decade.

Çayan et al. [5] conducted a meta-analysis involving 4473 patients with clinical varicocele and concluded that microsurgical subinguinal varicocelectomy resulted in higher natural pregnancy rates (mean, 41.97%; range, 33–50.9%), lower complication rates, and lower risk of recurrence compared to the laparoscopic, embolization, retroperitoneal, and macroscopic inguinal techniques. Other large-scale meta-analysis performed by Diegidio et al. [9] in 2011 found that microsurgical techniques are associated with higher natural pregnancy rates (mean, 44.75%; range, 33.8–51.5%) and reduced rates of hydrocele formation. More recently, Yuan et al. [10] published similar results in 2017, favoring microsurgical varicocelectomy in relation to open surgical techniques, especially due to higher natural pregnancy rates, lower incidence of postoperative complications, and shorter time to return to work. Esteves et al. [11] showed that infertile couples using assisted reproductive techniques, such as

intracytoplasmic sperm injection (ICSI), and whose male partner had undergone microsurgical varicocelectomy (Marmar technique) had a significantly higher rate of live births (46%) compared to those whose male partner had not undergone varicocele repair (31%), in addition to significant reduction in the risk of miscarriage (OR = 0.333; CI = 0.22–0.84; $p = 0.01$).

Although the effects of varicocele repair on spermatogenesis have been extensively studied, few studies have addressed its influence on androgen production. Since 1966, some authors have reported on the negative effects of hypoxia on Leydig cells and, consequently, on testosterone production [12]. Testicular biopsies in patients with varicocele revealed an increased number of Leydig cells, intracytoplasmic vacuolation, and reduction in the number of testosterone-positive Leydig cells [13]. Changes in Leydig cells in men with clinical varicocele have been associated with dysfunction of these cells and low androgen biosynthesis [6].

One of the most accepted theories is that varicocele-induced testicular hyperthermia inhibits 17-alpha-hydroxyprogesterone aldolase, an enzyme involved in the conversion of 17-hydroxyprogesterone to testosterone [14]. A more recent hypothesis is that in the presence of varicocele, free radicals exert a deleterious effect on Leydig cell mitochondria, inhibiting the expression and function of the steroidogenic acute regulatory protein (StAR), thus contributing to a reduction in testosterone [15].

In recent years, this topic has returned to the attention of researchers as improvements in health promotion and disease prevention contribute to increased life expectancy. Varicocele is a time-dependent disease and therefore has deleterious progressive effects on the production of testosterone [14, 16]. One can use exogenous testosterone in order to improve the serum levels, especially when there are associated symptoms of hypogonadism. It seems much more reasonable for the authors to try to improve the testosterone production by varicocelectomy instead of prescribing hormones to treat hypogonadism symptoms in the future if needed. Since this chapter is impelling the authors to discuss the role of varicocelectomy on testosterone production, we decided not to discuss other contraceptive methods, such as condom use and contraceptive pills. However, it is indeed very important to always inform the couple about all the contraceptive methods and discuss which is the better choice.

Many early studies reported contradictory findings on the effects of varicocele and its surgical repair on testosterone production. Some studies from the late 1990s showed no significant improvement of pre- and post-varicocelectomy testosterone levels [17], as well as a recent prospective study by Jangkhah et al. [18], in which 115 patients with varicocele grade 2 and 3 were compared with 240 healthy men, but no significant difference was found for adjusted testosterone levels after varicocele repair. The heterogeneity of the study populations and variations in preoperative hormone levels were considered as possible sources of bias in these studies.

Studies with better design were able to show results in favor of varicocelectomy with improvement of androgenic hormone levels. In a retrospective study, Hsiao et al. [19] stratified patients by age and observed that varicocelectomy for treatment of low testosterone levels (below 400 ng/dl) could also benefit older men. Zohdy et al. [20] conducted a prospective study and also found a significant improvement of hormonal levels after varicocelectomy in men with baseline levels of testosterone below 300 ng/dl (range, 379 ± 205.8 to 450 ± 170.2 ng/dl; $p < 0.0001$). In a case-control study, Tanrikut et al. [6] came to three important conclusions: (i) men with clinical varicocele have significantly lower testosterone levels than men without varicocele; (ii) microsurgical varicocelectomy increases testosterone levels, regardless of age, varicocele grade, and laterality; and (iii) varicocele is an independent factor of androgen deficiency, and its microsurgical repair may raise low preoperative levels of testosterone.

Recent studies support the findings that surgical repair of varicocele may result in improvement of androgenic hormone levels. Gomaa et al. [21] reported significant improvement of testosterone levels 6 months postoperatively in patients who underwent microsurgical repair of clinical varicocele (grade 1 to 3) compared with patients with varicocele who chose not to undergo surgi-

cal treatment. In 2017, Chen et al. [21] conducted a meta-analysis of seven studies involving a total of 712 patients who had undergone varicocelectomy and found that the mean serum testosterone level in the postoperative period increased by 34.3 ng/dl (95% CI, 22.57–46.04; $p < 0.001$, $I^2 = 0\%$) compared with preoperative levels. Varicocelectomy significantly improved testosterone in hypogonadal subfertile men, showing that the surgical treatment of varicocele may contribute to maintain healthy levels of androgens in subfertile men for a period of 6 to12 months following surgery [22].

Surgical Approach

Based on the knowledge acquired in recent years on the negative impact of clinical varicocele on androgen hormone production, the surgical repair of varicocele may soon become a treatment option offered by urologists to hypogonadal patients. This specifically brings up some important issues, such as the occurrence of asymptomatic hypogonadism and the willingness of patients to perform vasectomy.

Vasectomy is commonly performed through scrotal incisions; the vas deferens is ligated to the deferential artery and deferential vein, thereby damaging one of the three testicular arterial supplies and an important pathway of venous return. In this case, microsurgical varicocelectomy would further jeopardize testicular irrigation if an inadvertent ligation of the testicular artery occurs, leading to tissue hypoxia and probably exacerbation of hypogonadism.

Surgeons are often compared to artists, especially when they use their imagination to overcome difficulties in surgical practice. Thus, with a touch of creativity, other surgical approach may be proposed, involving a single incision to perform vasectomy and left varicocelectomy, aiming at achieving the contraceptive effect and improvement of testosterone production within the same operating time. In this case, a subinguinal incision is preferred, beginning with the dissection of the anterior compartment of the spermatic cord and ligation of varicose veins under microscopic vision, preserving the testicular artery and lymphatic vessels. Next, the posterior compartment is dissected, with isolation of the vas deferens and preservation of the deferential artery and deferential vein. Following, the ligation of only the vas deferens is made with nonabsorbable suture and, preferably, interposing a fascia between the stumps. Thus, as a final result of this surgical technique, the varicose vessels were treated, the vas deferens was ligated, and all arteries and also the deferential vein were preserved to achieve maximum tissue oxygenation as well as oxidative stress reduction, creating a favorable environment for androgen production (Figs. 51.1 and 51.2). It is important to state that this surgical

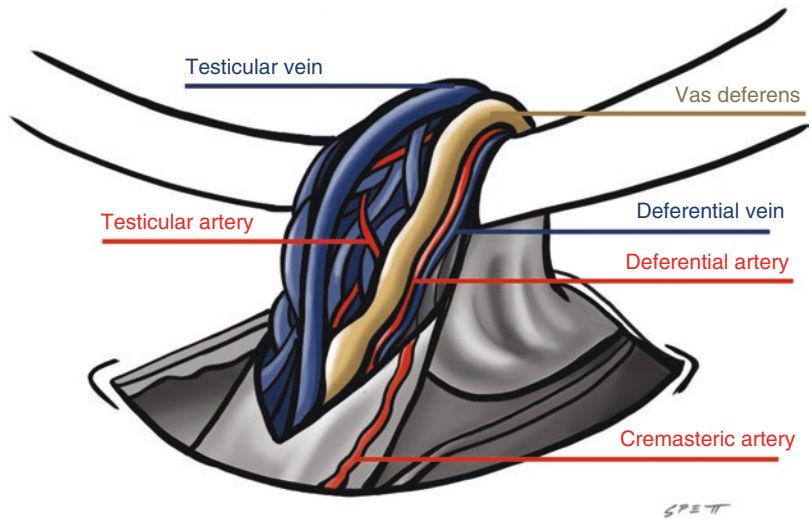

Fig. 51.1 This figure shows the spermatic cord suspended by a Penrose drain with its anatomical contents: veins, arteries, and the vas deferens

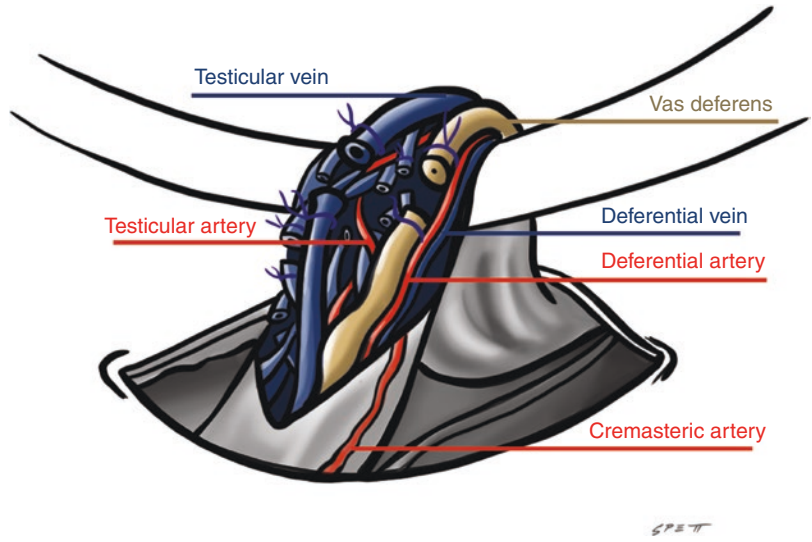

Fig. 51.2 This figure shows the surgical approach suggested by the authors: a microsurgical technique performing ligation of all varicose veins preserving the testicular and cremasteric arteries and the ligation of vas deferens preserving the deferential artery and deferential vein, optimizing tissue oxygenation, and increasing testosterone production

approach was first described by Lee et al. in a published article in 2007 titled "Simultaneous Vasectomy and Varicocelectomy: Indications and Technique" [23].

Conclusion

The theoretical knowledge of pathophysiology of varicocele in androgenic hormonal balance, testicular vascularization, and vasectomy and varicocelectomy techniques allow the physician to propose the best treatment to the patient, aiming at obtaining a contraceptive method and promoting the homeostasis necessary for improving testicular hormone production.

Review Criteria

A search of studies was conducted examining the relationship between varicocele repair and improvement in androgenic hormonal levels as well as surgical male contraception using search engines such as PubMed, MEDLINE, and ScienceDirect. The search period occurred between April 2018 and May 2018. The following keywords were used to search for studies and data: "varicocele," "varicocelectomy," "varicocele repair," "infertile men," "male infertility," "testosterone," "androgen deficiency," "family planning," and "vasectomy." Articles published in languages other than English were not considered. Data that were solely published in conference or meeting proceedings, websites, or books were not included. Book chapter citations provide conceptual content only.

Multiple Choice Questions and Answers

1. Which of the following complications is not commonly associated with vasectomy?
   (a) Chronic scrotal pain.
   (b) Infection.
   (c) Hematoma.
   (d) **Hydrocele.**
2. Of the varicocele correction techniques listed below, which is the most effective and has the lowest risk of complications?
   (a) Retroperitoneal approach.
   (b) Inguinal approach.
   (c) Laparoscopic approach.
   (d) **Microscopic subinguinal approach.**

3. Regarding the relationship between clinical varicocele and testosterone production, which of the following are correct statements?
   I. Correction of clinical varicocele in hypogonadal patients may improve testosterone levels.
   II. The presence of clinical varicoceles is an independent factor for androgen deficiency.
   III. Testicular hyperthermia potentiates the action of 17-hydroxyprogesterone aldolase.
   IV. Varicocele repair in older men can also improve testosterone levels.
   V. There is no relationship in the literature between varicocele and testosterone production.
   (a) I, II, III, IV.
   (b) **I, II, IV.**
   (c) II, III, IV, V.
   (d) I, II, V.
4. When varicocele repair and vasectomy are performed at the same session, what would be the ideal technique to perform the procedure according to the authors?
   (a) **Single microsurgical subinguinal incision.**
   (b) Subinguinal and conventional scrotal incisions.
   (c) Retroperitoneal approach and conventional scrotal incision.
   (d) Embolization and conventional scrotal incision.
5. All the following are expected outcomes of varicocele repair except:
   (a) Improved sperm motility.
   (b) **Increased risk of multiple gestation.**
   (c) Improved sperm counts.
   (d) Elevated serum testosterone.

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52 Symptomatic Male with Subclinical Varicocele Found on Ultrasound Evaluation

Ahmad Majzoub

Key Points

- Subclinical varicocele is defined as venous reflux in the internal spermatic vein without palpable distention of the pampiniform plexus.
- Pain in the context of varicocele is believed to arise secondary to hydrostatic pressure elevation and venous dilatation resulting in Wallerian degeneration in the adjacent nerve fibers and intra-testicular hypoxic injury.
- Varicocele pain is an uncomfortable, dull ache in the scrotum. This ache is the result of blood pulling in the scrotum that gets worse after prolonged standing or strenuous exercise and gets better after lying down.
- Conservative treatment is preferred as an initial approach to manage symptomatic patients with subclinical varicocele.
- Varicocelectomy is not indicated in patients with subclinical varicocele.
- Spermatic cord denervation can be considered in patients with chronic scrotal content pain provided that a favorable response to spermatic cord block was achieved.

Introduction

Throughout urology practice, scrotal pain remains a frequently encountered problem. It is dominant in about 2–10% of patients with varicocele [1]. The effect of varicocele on pain is relatively little investigated when compared to its connection with infertility. Surgical treatment to restrict pain was first explored by Biggers and Soderdahl to a clinical varicocele in 1981. Pain had an improvement rate of 48% [2]. Later studies showed that the rate was actually higher [3], granting surgery an advantage in such cases.

Historically, management of subclinical varicocele has been controversial. It lacks a unified global definition regarding its principal entity. Comhaire and Kunmen introduced possibly the most acceptable definition by stating that it is venous reflux in the internal spermatic vein without palpable distention of the pampiniform plexus [4]. Though this is to a great extent described as an incidental scrotal ultrasound finding, studies on subclinical varicocele mainly investigated the value of its management in infertile men [5]. In this chapter, we endeavor to arrive at the best practice that should be employed to manage a symptomatic male with subclinical varicocele.

A. Majzoub (✉)
Department of Urology, Hamad Medical Corporation, Weill Cornell Medicine Qatar, Doha, Qatar

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Pathophysiology of Pain in Varicocele

Varicocele is thought to be caused by the presence of incompetent or absent valves within the veins of the testis. Accordingly, blood may flow backward causing veins to dilate. Anatomical differences may contribute to its etiology since it is predisposed on the left side. As a result, venous hydrostatic pressure elevates leading to harmful effects of varicocele on male fertility. Furthermore, elevation in hydrostatic pressure is considered to be a main factor in pain development. It causes venous dilatation followed by compaction of the adjacent nerves along the spermatic cord. Parekattil et al. demonstrated Wallerian degeneration in his attempt to find a potential anatomical basis for denervation procedures [6]. Wallerian degeneration is a process that results when a nerve fiber is cut or crushed and the part of the axon distal to the injury degenerates. The location of those nerves was along the cremasteric muscle fibers, perivasal tissues, and perivessel tissues [6]. Intra-testicular stasis along with hypoxic injury is yet another consequence of elevated hydrostatic pressure that can result in pain [7]. Carefully designed clinical studies are still required for validation regardless of their hypothetical plausibility. Otherwise, they remain as theories that need verification.

Clinical Presentation and Diagnosis

Clinical Scenario

A 32-year-old patient presents to clinic with left hemiscrotal pain that is localized to the testicle for more than 3 months duration. The pain is experienced as compressing in character, occurring about 2–3 times per week for few hours, and is aggravated by physical activity and relieved by rest. The patient's clinical examination fails to detect any abnormal finding with no tenderness elicited during testicular or epididymal palpation. The ultrasound findings note reflux with Valsalva in the left pampiniform plexus of veins with a maximum venous diameter of 2.4 mm.

Since chronic orchialgia, defined as testicular pain of more than 3 months duration, could be secondary to a wide scheme of etiologies, it is crucial to thoroughly understand the different presentations that patients suffer from. Generally speaking, varicocele pain is an uncomfortable, dull ache in the scrotum. This ache is the result of blood pooling in the scrotum. It is a pain that gets worse after prolonged standing or strenuous exercise and gets better after lying down. The relief after lying down is due to the draining of blood from the scrotum. Proper physical examination is the one preformed in a warm atmosphere to ensure cremasteric relaxation in both standing and supine positions.

Venus dilatation is commonly measured using duplex ultrasonography. While radiologic imaging for evaluating varicocele is feasible, it is not compulsory. The appropriate criteria for subclinical varicocele diagnosis are still controversial. Nevertheless, demonstration of retrograde flow during Valsalva along with a spermatic vein diameter >3.0 mm is for the most part congruous with clinical varicocele [8]. Palpation is possible when the diameter of internal spermatic veins measures 3.0–3.5 mm. On the other hand, reversal of flow is manually felt in veins >3.5 mm in diameter [9]. Diagnosis of varicocele is not limited to ultrasonography as various other radiographic examinations exist—spermatic venography, radionuclide scanning, and thermography, to name a few. Yet being invasive and of high cost made them less appealing and barely put to use.

Differential Diagnosis

Accurate patient evaluation remains a key factor in diagnosis while ruling out many other conditions. Testicular torsion or tumor, epididymitis, inguinal hernia, hydrocele, and spermatocele are potentially serious etiologies that must be addressed before attributing orchialgia to varicocele. Testicular pain may also arise from other somatic or visceral source. Thus, investigating and exploring urinary tract symptoms, distal ureteral stone, and irritable bowel syndrome are warranted.

A state of perplexity is dominant when subclinical varicocele is the only pathology. Orchialgia

is considered idiopathic when there is no direct or obvious cause to its chronic existence. This is the case in 25% of patients [10].

Treatment Options

Conservative/Nonsurgical Options

A number of painful scrotal conditions are managed with conservative/nonsurgical options. Men with chronic orchialgia as well as clinical varicocele-related pain are no exception. It is always beneficial to start treatment with scrotal support, nonsteroidal anti-inflammatory medications, and limited physical activity.

In military population with varicocele, a success rate of 0.04% was noted upon utilizing only conservative measures [3]. Another study was performed on 140 patients undergoing conservative treatment for up to 8 weeks. Only 5 patients (3.5%) showed partial improvement followed by later recurrence [11]. Though studies have issued shy results which act against adopting conservative measures, still opting to go for such initial approach in treatment is of no harm. Nevertheless, the vast majority of men with varicocele-related pain undergoing conservative treatment will eventually require surgery.

Varicocelectomy

Surgical intervention is recommended when it comes to clinically palpable disease (grades 1, 2, or 3) [12]. Therefore, all studies assessing the value of varicocelectomy for painful varicoceles have eliminated subclinical disease. The majority of these trials have been retrospective in design and included small number of cases [13]. Furthermore, studies comparing the worthiness of different techniques of varicocelectomy for pain resolution remain nonexistent [13].

It can be stated that the grade of a painful varicocele might have an influence over the repair outcome. Monitoring 82 patients over a period of at least 3 months following microsurgical varicocelectomy showed complete resolution of pain in 88% and no response in 11% of patients. Most importantly, among the group of non-responders, grade 3 varicocele was present in 6.1%, grade 2 in 3.6%, and grade 1 in 1.2% [11]. Furthermore, an extended follow-up (1 year), according to another study by Kim et al., resulted in complete or marked resolution in 91.2% of patients [13].

Despite an obvious benefit in patients with clinically palpable varicocele, the lack of reports evaluating the effect of surgery on patients with subclinical varicocele that is not included in the list of surgery indications of all major guidelines leaves this approach unattractive.

Spermatic Cord Denervation

Microsurgical denervation of the spermatic cord is a technique that has been proven both effective and safe when it comes to management of chronic orchialgia [14, 15]. Over the last two decades, many articles have been published in its favor. It was first introduced in the year 1978 with a case report of two patients [14]. Some urologists linked microsurgical denervation of the spermatic cord to varicocelectomy, thus defining it as an "extended varicocelectomy." Unlike varicocelectomy, which is the transection of internal and external spermatic veins leaving other cord structures intact, spermatic cord denervation is carried out by transecting all spermatic cord structures, preserving arterial inflow to the testicle and several lymphatics to prevent postoperative testicular atrophy and hydrocele formation. The target being to interrupt neural pathways to and from scrotal contents inhibiting afferent nerve stimulation and downregulating pain centers. Subclinical varicocele remains unfit for such a procedure for it is directed toward idiopathic chronic orchialgia. Moreover, a great benefit proven in favor of microsurgical denervation is its foreseen effect through the upfront use of a simple cord block. For instance, a positive spermatic cord block specifies a later success rate of microsurgical denervation ranging between 70% and 97% [16, 17].

Subinguinal microsurgical spermatic cord denervation was performed on 52 testicular units belonging to 50 patients according to a recent

study by Marconi et al. [15]. A 6-month follow-up of patients after surgery has been performed. Results marked pain completely resolved in 40 (80%) patients, intermittent testicular discomfort persisted in 6 (12%) patients, and no change in pain severity in 4 (8%) patients.

Conclusion

Attempting a conservative approach in the management of pain and treatment of patients with orchialgia and a radiographic finding of a subclinical varicocele is always preferred initially. Additionally, subclinical varicocele is not an indication for surgical options such as varicocelectomy or spermatic cord denervation. Nevertheless, when surgery is to be considered, it should mind the algorithm for chronic scrotal content pain. In that context, spermatic cord denervation may be an option. A favorable outcome is marked by transient symptom relief of a cord block. Failure to achieve any improvement after the cord block yields pain management a potential alternative choice.

We still need to better understand chronic scrotal pain. Further research will certainly help us determine whether subclinical varicocele significantly contributes to pain or it being a mere coincidental finding. As of current evidence, the fact remains that surgical intervention to a subclinical varicocele is not suggested for scrotal content pain.

Review Criteria

An extensive search of the literature of the association of orchialgia with subclinical varicocele was done using scientific search engines including PubMed, MEDLINE, ScienceDirect, and Google Scholar. Search criteria included the following keywords: "varicocele," "subclinical," "orchialgia," "testicular pain," "varicocelectomy," and "cord denervation". Data from published papers or book chapters were included.

Multiple Choice Questions and Answers

1. Subclinical varicocele is defined as:
   (a) Dilatation of the spermatic veins that is only felt with Valsalva maneuver.
   (b) Ultrasound demonstration of retrograde flow during Valsalva along with a spermatic vein diameter >3.0 mm.
   (c) Ultrasound demonstration of retrograde flow during Valsalva along with a spermatic vein diameter >3.5 mm.
   (d) **Venous reflux in the internal spermatic vein without palpable distention of the pampiniform plexus.**
   (e) A varicocele that does not have a clinical impact on the patient.
2. Pain secondary to varicocele is explained by the following pathophysiologies except:
   (a) Venous hydrostatic pressure elevation.
   (b) Intra-testicular stasis.
   (c) Intra-testicular hypoxia.
   (d) Wallerian degeneration of the adjacent spermatic nerves.
   (e) **Testicular atrophy.**
3. A symptomatic subclinical varicocele is best managed by:
   (a) Venous embolization.
   (b) Laparoscopic varicocelectomy.
   (c) Microsurgical varicocelectomy.
   (d) **A trial of conservative treatment with NSAIDs, scrotal elevation, and rest.**
   (e) Ice packs.
4. Select the false statement:
   (a) Varicocelectomy is only recommended in clinically palpable varicocele.
   (b) Varicocele grade might have an influence over the repair outcome.
   (c) The lack of reports evaluating the effect of surgery on patients with subclinical varicocele rendered this approach unattractive.
   (d) **Conservative treatment is highly successful in alleviating chronic scrotal pain.**
   (e) Accurate patient evaluation remains a key factor in diagnosis of chronic orchialgia while ruling out many other conditions.

5. Microsurgical spermatic cord denervation:
   (a) Entails cutting only the spermatic nerves under higher magnification leaving all cord contents intact.
   (b) **Has a success rate between 70% and 97% provided that a positive spermatic cord block has been achieved.**
   (c) Is indicated in patients with subclinical varicocele.
   (d) Is always accompanied with a vasectomy.
   (e) Can be performed radiographically.

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Recurrent Grade 2/3 Varicocele After Microsurgical Varicocelectomy and Abnormal Semen Parameters in a Couple Attempting Conception for >3 Years

Jorge Hallak, João Arthur Brunhara, and Thiago Afonso Teixeira

Key Points

- Even though recurrence of varicocele is often related to the surgical technique and access employed, a number of other anatomical peculiarities could explain the persistence of varicocele after varicocelectomy.
- Internal spermatic vein reflux plays a critical role in recurrent varicocele.
- Studies evaluating the treatment of recurrent varicocele after a microsurgical initial treatment are scarce, and when available, they are usually uncontrolled and represent a small part of a heterogeneous population.
- Based on the best evidence available, choosing an optimal method for treatment of clinical recurrent varicocele after microsurgical varicocelectomy is not possible and randomized controlled trials must be done.
- Author's suggestion for recurrent clinical varicocele after microsurgery and abnormal semen parameters is to redo microsurgical varicocelectomy, using an intraoperative Doppler ultrasound, with an incision as low as possible by an experienced microsurgeon.

J. Hallak (✉)
Androscience – Science and Innovation Center and High-Complexity Clinical and Research Andrology Laboratory, Sao Paulo, Brazil

Men's Health Study Group, Institute for Advanced Studies, Division of Urology, Reproductive Toxicology Unit, Department of Pathology, University of São Paulo, Sao Paulo, Brazil
e-mail: hallakj@androscience.com.br

J. A. Brunhara
Androscience – Science and Innovation Center and High-Complexity Clinical and Research Andrology Laboratory, Sao Paulo, Brazil

Men's Health Study Group, Institute for Advanced Studies, Division of Urology, University of Sao Paulo, Sao Paulo, Brazil

T. A. Teixeira
Androscience – Science and Innovation Center and High-Complexity Clinical and Research Andrology Laboratory, Sao Paulo, Brazil

Men's Health Study Group, Institute for Advanced Studies, Division of Urology, University of Sao Paulo, Sao Paulo, Brazil

School of Medicine, Federal University of Amapa, Macapa, Brazil

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Introduction

> "*A puzzle inside a riddle wrapped in an enigma*"
> *(Sir Winston Churchill)*

The word "varicocele" is derived from a combination of two ancient languages, from Latin, *varix* (tortuous blood flow), and from Greek, *kele* (edema). Although the innumerous links between varicocele and male infertility have been recognized for many centuries and have inspired andrologists to uncover this multifaceted real problem for men's sexual and reproductive health, the introductory phrase of this chapter by Prime Minister Winston Churchill of Great Britain to President Franklin D. Roosevelt of the United States in a political referral to Premier Joseph Stalin of the Soviet Union, at the Yalta Conference in Crimea during the World War II, could only be feasible as they foresee the "light at the tunnel" to end the war. In our understanding, varicoceles fit perfectly into this phrase, but on the contrary, basic scientists and clinicians that deal with infertility and hypogonadism are far from foreseeing the "light at the end of this tunnel" to have a final decision upon every aspect of venous reflux to the pampiniform plexus. Using a metaphor in fact, sometimes one is not even capable to identify where is the "entrance for the varicocele tunnel," whether it is the correct medical diagnosis, the role of the varicocele amid other medical conditions and chronic diseases of the infertile male (diabetes, metabolic syndrome, alcohol, tobacco, drugs, medications, sedentarism, environmental pollutants, etc.), what are the correct validated sperm functional tests to be applied (ROS, DNA fragmentation, whether SCSA, TUNEL, or COMET), proper microsurgical treatment followed or not by antioxidants or other medical therapy, the debate of varicocelectomy in the era of in vitro fertilization (IVF), and last but not least, what to use as measurement for success: pregnancy rates, sperm quality improvement, successful rates in assisted reproductive technology (ART), cost-effectiveness, or the role of varicocele in male hypogonadism. Therefore, the varicocele dilemma will still be a motivation for countless discussions and scientific discoveries for decades to come. Part of this eternal dilemma relies on the fact that the current classification system is over 50 years old, and although the definition of varicocele includes the existence of venous reflux, classification only stands for vein dilation of the pampiniform plexus into three major clinical categories. It is time for a change [1].

The present chapter is about the theoretical scenario of a recurrent grade 2/3 varicocele after microsurgical varicocelectomy and abnormal semen parameters in a couple attempting conception for more than 3 years. I confess (JH) that even after more than 20 years in practice and having performed literally thousands of varicoceles myself and hundreds more helping residents, such a case is unknown to my memory. What we indeed have seen is either the ultrasonographical persistence or recurrence, the immense majority without laboratory, seminal or clinical repercussion, but definitely not to the extent of becoming again grade 2 or 3 clinically relevant. Anyway, we will discuss hypothetically how to deal with this situation.

Anatomy and Pathophysiology

In order to understand the proposed pathophysiology of varicocele recurrence, it is important to consider the testicular vascular anatomy as well as the vascular supply to the pelvic region and drainage to and from the retroperitoneum.

The arterial supply to the testis has three major participants: the testicular artery or arteries, the cremasteric artery or arteries, and the artery of the vas deferens. The testicular artery is the main source for oxygen-rich blood supply, but one cannot underestimate the role of the cremasteric artery and collateral perfusion of the testis in stressful situations (testicular atrophy, hypotrophy) or in the event of an iatrogenic ligation of the main testicular blood supply in inguinal or subinguinal surgical procedures like hernia repair or open classical varicocelectomy. Return of venous blood from the testis is guaranteed by the pampiniform plexus, which drains to the internal spermatic cord (testicular), vasal (deferential),

and external spermatic cord veins (cremasteric). The fact that palpable varicosities in the spermatic cord are only detectable around the age of puberty leads to the observation of an increase in blood supply during the onset and developing of puberty, from Tanner stages I to V, making veins previously undetectable visible with overperfusion and diminished blood return to central veins, presenting concomitant ectasia and thus becoming clinically relevant.

Anatomic studies revealed the presence of left-to-right venous communication system, situated superficially as well as in the deep drainage system, located at the ureteric, L3 to L5, spermatic, scrotal, retropubic, saphenous, sacral, and pampiniform plexuses [2].

The left spermatic vein subsidiaries at the level of L4 in quite all men drain into medial and lateral divisions at this particular level of the lumbar vertebrae. That is why surgical procedures done above this level have a higher chance of failure, due to multiple divisions of the spermatic venous system [3].

The question here in relation to the case proposed for discussion at this present chapter is that it is unlikely that any microsurgical procedure conducted by a trained urologist be performed in such a high anatomical level much above the inguinal canal. The explanation for recurrence in this hypothetical scenario would be not even of an inguinal microsurgical varicocele repair but of an even higher incision done by improper, untrained hands and less likely, but possible, due to anatomical peculiarities of the spermatic veins or due to the entrance of retroperitoneal veins below the level of the initial incision at the spermatic cord. Even though recurrence of varicocele is often related to the surgical technique and access employed, a number of other anatomical peculiarities could explain, in some cases, the persistence of varicocele after varicocelectomy. For example, the role of cremasteric veins and external spermatic veins – which cannot be ligated during the laparoscopic or open retroperitoneal approach – has been debated, and it has been hypothesized that cremasteric reflux could be a relevant factor for recurrent varicocele. However, Franco et al. have demonstrated with a venographic approach that from 19 patients presenting with recurrent varicocele, none presented reflux via extra funicular veins and all presented internal spermatic vein (ISV) reflux [4]. The authors further concluded that recurrent varicocele is a disease concerning ISVs [4, 5].

In fact, series in which venography was consistently performed in patients with recurrent varicocele have shown a critical role for the ISVs, with redundancies and duplications playing a major role and collateral veins being less frequent. In a series of 17 patients undergoing venography for recurrent varicocele, Sze et al. have shown that two thirds of patients presented duplication and redundancy of the gonadal vein draining into a single vein, with such duplications mostly confined to the inguinal canal and pelvic region [6]. Collateral veins draining to other retroperitoneal veins – such as renal, lumbar, and iliac veins – were found in a minority of patients. Another study with 33 patients undergoing venography for recurrent varicocele attributed recurrence to a duplication of the gonadal vein in 66% of cases, only more frequently in the midportion of the vein rather than the inguinal and pelvic portions [7].

Although gonadal vein duplication seems to be the most important anatomical factor for recurrent varicocele, collateral drainage should be considered in such cases. Rais-Bahrami et al. have shown that patients undergoing salvage embolization of varicocele were more likely to present inguinal collaterals and retroperitoneal collaterals than subjects undergoing primary embolization. A proof of the mechanisms has been discussed in the previous paragraph [8].

Choice of Surgical Technique

Open microsurgical technique for inguinal or subinguinal varicocelectomy led to the fewest recurrence rates found in the literature, probably because of the higher magnification of spermatic veins, which improves the ability of the surgeons to visualize and ligate all [9], and to the fact that the incision is subinguinal, located one centimeter lower or even more than the external inguinal

canal. The overall recurrence rate of microsurgical approach, including subinguinal and inguinal techniques, was 1.05% (0–3.57%) [9–17].

In a meta-analysis, Cayan et al. analyzed randomized controlled trials that compared different approaches (microsurgical, open non-microsurgical, and laparoscopic) of varicocelectomy in infertile men with clinically palpable varicocele (G2/G3). The incidence of recurrent varicocele for microscope technique was 1.9% (10 in 518 cases) [18]. In another meta-analysis, Wang et al. established the inguinal and subinguinal microscopic varicocelectomy as the optimal management of varicocele mainly because it had the best outcomes with significant increases in semen parameters, the highest pregnancy rates with low rates of complication, including recurrence [19].

Microscopic subinguinal redo varicocelectomy was normally chosen as the approach for recurrent varicocele when the first technique used was a non-microscopic one, and this repair improves postoperative semen parameters and natural pregnancy rates and also decreases the need for use and level of assisted reproductive technology (ART) when compared with observation only [20]. Significant predictive factors contribute to the success of redo varicocelectomy in infertile men with recurrent varicocele and no improvement of semen quality after the initial procedure, such as serum follicle-stimulating hormone (FSH) concentrations <14.6 mIU/ml, peak retrograde flow (PRF) <37 ml/s on Doppler ultrasound, testicular volume >27.3 cc preoperatively, number of ligated veins >6, and time to recurrence >10 months [21].

Studies evaluating the treatment of recurrent varicocele after an initial microsurgical treatment are scarce, and when available, they are usually uncontrolled and represent a small part of a heterogeneous population. Grober et al. studied 54 men who were submitted to a subinguinal microsurgical varicocelectomy for varicocele recurrence. Just 4% of their population underwent microsurgical inguinal varicocelectomy as initial treatment. Mean testicular volume, mean serum testosterone, and sperm parameters all improved significantly after the redo procedure, without clinical recurrences as outcome and with an overall pregnancy rate of 40% [22]. Based on the best evidence available in medical literature, choosing an optimal method for treatment of clinical recurrent varicocele after microsurgical varicocelectomy as initial treatment is not possible and randomized controlled trials must be done to resolve this question properly. Whether is the grade 3 varicocele more prone to recurrence or the grade 2, or if it is not dependent on grade, but in other variables, like number and diameter of veins ligated or left without ligation as they were considered by the surgeon too small to consider ligation and afterward increased in diameter and started to present reflux is still a matter to be clarified. We could start a debate about the etiology of venous reflux in order to try to make hypothesis for these questions. In 1966, Ahlberg proposed that testicular veins contained valves that were absolutely necessary for the protection of the testis against the harmful effects of venous reflux and the lack or incompetence of them caused left-sided varicoceles, but not right-sided ones, after he found absence of valves in 40% of postmortem left spermatic veins as compared to 23% in right-sided ones [23]. However, the controversy arouses when 26.2% of patients with a competent valve system still presented with clinical varicocele, according to radiographic studies [24]. So one possible explanation for the recurrence of grade 2/3 varicoceles could be that in the moment of microsurgery, a few veins that were with smaller diameter, with less than, let's argue, 2–3 mm, that were purposely or inadvertently left over or belonged to the posterior plexus within the spermatic cord, that were more difficult to identify for the unexperienced surgeon or resident in training, or that either were not considered relevant or were simply missed even by the more experienced microsurgeon had the same endothelial and muscle valve characteristics as the ones initially presented with reflux or incompetence. Hydrostatic pressure mechanisms started to play their role in the upright standing position (gravity), and after months or even years, these veins gained diameter. And as they had the same defective inner conditions, therefore, recurrence was on its way.

Other possible etiological hypothesis for the recurrence of a varicocele could be theoretically explained by a left-to-right venous communication system, situated superficially as well as in the deep drainage system, as previously pointed in the text, in the common scenario where the initial surgical procedure was only performed in the left-sided clinical varicocele and the subclinical right-sided varicocele was left untouched and with time these communication systems located at the ureteric, L3 to L5, spermatic, scrotal, retropubic, saphenous, sacral, and pampiniform plexuses could develop into bigger diameter refluxive veins and became relevant again. This is purely an anatomical question and has nothing to do with surgical skills or technique. The question for correcting subclinical varicocele is a matter of debate, although the European Association of Urology and American Society of Reproductive Medicine guidelines do not recommend fixing subclinical varicoceles [25, 26]. Others have found no improvement in pregnancy rates after repair of subclinical varicoceles. Some studies demonstrated the benefit of correcting a right subclinical varicocele in the presence of a left clinical varicocele [27]. To answer this hypothesis, one would have to look back at the initial surgical procedure and check if there was a right-sided varicocele that was not fixed.

Also, after performing literally many thousands of microsurgical varicocelectomies over the last 20 years, we can argue that in many tough intraoperative circumstances, like excessive fibrous tissue in the spermatic cord, redo operations, obesity, excessive fat tissue within the spermatic cord, or residents in training, the use of an intraoperative Doppler ultrasound is useful and guides the surgeon toward a more effective and less risky procedures, as multiple spermatic arteries can be identified in approximately 40% of the spermatic cords during microsurgical varicocelectomy at the subinguinal level [28, 29]. The identification of the main spermatic artery can be confirmed by visualization of clear pulsatile movement and/or evidence of antegrade, pulsatile blood flow after gentle lifting and partial occlusion of the vessel. However, the identification of tiny secondary arteries is not always obvious, and intraoperative Doppler ultrasound has been used for this purpose; however, the first description of systematic use of intraoperative Doppler ultrasound during microsurgical subinguinal varicocele repair was described by our group [30]. A significant higher number of arteries were identified and preserved when intraoperative vascular Doppler was used. Data concerning surgery using the sonographic device and without using it showed that a solitary artery was identified in 45.5% and 69.5% of cords, respectively, while two arteries are identified in 43.5% and 28.5%, respectively, and three or more arteries are identified in 11% and 2%, respectively. Also, the average number of internal spermatic veins ligated was significantly higher in the Doppler group. Accidental artery ligation documented by a pulsatile twitching of the ligated vessel stump under magnification occurred in two cases in which the Doppler was not applied [30]. So one can assume that in the absence of an intraoperative Doppler ultrasound, some veins are more likely to be missed and could develop into a recurrence. In one of the authors' experience (JH), the Doppler ultrasound proves to be very useful in the following circumstances: initial couple of hundred microsurgeries, fibrotic spermatic cord, redo varicocelectomies, previous hernia repair, previous manipulation of the spermatic cord for any reason, and very important, to teach and guide residents in training for the often-tricky cat-rat search for the spastic and contracted testicular artery after initial manipulation by training hands. A good advice is to keep Doppler ultrasound handy in the surgical room, and in the case of any intraoperative difficulties that happen, it is immediately available.

Finally, we have quickly looked at our own data regarding recurrence of clinical varicoceles and redo varicocelectomies and could localize exactly ten patients that were resubmitted to a second microsurgical varicocele procedure in the universe of approximately 2690 varicocelectomies, performing a total of almost 5000 varicocele units (left, right), with a clinical recurrence rate of approximately 0.40%. We do not have, at this moment, the data regarding the Doppler ultrasound persistence of reflux, in 3, 6, and 12 months

postoperative sonographic controls, which is statistically higher but clinically insignificant.

In conclusion in the hypothetical case presented here, we would redo the microsurgical varicocele with the use of an intraoperative Doppler ultrasound and use an incision as low as possible.

Review Criteria

A search of studies examining the relationship between recurrent clinical varicocele after microsurgery and abnormal semen parameters was performed using search engines such as PubMed, MEDLINE, and Web of Science. The start and end dates for these searches were January and December 2018, respectively. The overall strategy for study identification and data extraction was based on the following keywords: "recurrent clinical varicocele," "abnormal semen parameters," "infertility," and "microsurgical varicocelectomy." Just articles published in English were considered.

Multiple Choice Questions and Answers

1. Which technique for varicocelectomy led to the fewest recurrence rates found in the literature nowadays?
   (a) **Open microsurgical subinguinal approach.**
   (b) Open non-microsurgical subinguinal approach.
   (c) Open non-microsurgical inguinal approach.
   (d) Laparoscopic approach.
2. The oxygen-rich blood supply to the testis has the following participant arteries, except:
   (a) Testicular artery.
   (b) Cremasteric artery.
   (c) **Artery of the epididymis.**
   (d) Artery of the vas deferens.
3. Significant predictive factors that contribute to the success of redo varicocelectomy on infertile men with recurrent varicocele and no improvement of semen quality after the initial procedure are the following, except:
   (a) **LH serum concentration of <14.6 mIU/ml.**
   (b) Testicular volume >27.3 cc preoperatively.
   (c) Number of ligated veins >6.
   (d) Time to recurrence >10 months.
4. An intraoperative Doppler ultrasound in varicocelectomy proves to be useful in the following circumstances, except:
   (a) Fibrotic spermatic cord.
   (b) Redo varicocelectomies.
   (c) **Presence of cysts of spermatic cord.**
   (d) Previous hernia repair.
5. For a couple attempting conception for >3 years presenting a recurrent clinical varicocele after microsurgical varicocelectomy and abnormal semen parameters, which treatment could be indicated?
   (a) **Microsurgical open varicocelectomy, the use of an intraoperative Doppler ultrasound, and an incision as low as possible.**
   (b) Laparoscopic varicocelectomy.
   (c) Non-microsurgical open varicocelectomy with the use of an intraoperative Doppler ultrasound and use an incision as lower as possible.
   (d) Microsurgical open approach without the use of an intraoperative Doppler ultrasound using a high inguinal incision.

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Grade 2/3 Varicocele in a 15-Year-Old Healthy Boy with Normal Testis Volume (and Normal Semen Analysis) with a History of Varicocele and Infertility in His Father

Nicola Zampieri, Simone Patanè, Federica Bianchi, Marta Peretti, and Francesco Saverio Camoglio

Key Points

- Varicocele is a pathologic condition with a typical peripubertal onset with different reported incidence in the literature. It usually affects 15–20% of the young males. This condition is potentially associated with reduced fertility in males, and it is often related to pathologic alteration of semen.
- Correlation between varicocele and male infertility is strong. This condition is today the main cause of pathologic alterations of the semen. This important association has led to an increased interest of the scientific community toward the epidemiological aspects of varicocele.
- Somatometric parameters are related with varicocele. The most important is the body mass index (BMI). The correlation is especially evident for adolescents and not for adults.
- The majority of the studies in the scientific literature have shown an important correlation between physical activity, development of varicocele, and consequent semen alterations.
- Varicocele is the most common identifiable cause of male subfertility, and it is well known its correlation with testicular growth arrest.

History of Varicocele in Peripubertal Age

Varicocele is a pathologic condition with a typical peripubertal onset with different incidence among the several authors. It usually affects 15–20% of the young males. This condition is potentially associated with reduced fertility in males, and it is often related to pathologic alteration of semen.

Varicoceles may be classified by size into grades 1 to 3 or small, medium, and large. Small varicocele (grade 1) may be evident only during Valsalva maneuver. Medium-size varicoceles (grade 2) are palpable without Valsalva maneuver, and large varicoceles are visible as a scrotal space-occupying lesion.

N. Zampieri (✉) · S. Patanè · F. Bianchi · M. Peretti
F. S. Camoglio
Department of Pediatric Surgery, Woman & Child Hospital, Department of Surgery, Dentistry, Paediatrics and Gynecology, Division of Pediatric Surgery, University of Verona, Italy, Verona, Italy
e-mail: dr.zampieri@libero.it

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Camoglio et al. [1] have demonstrated that in peripubertal age, grade 1 varicocele predominates with 45.8%, while grade 2 and grade 3 have an incidence of 29.4% and 24.7%, respectively.

Usually, varicocele is asymptomatic, but sometimes patients may present with symptoms such as scrotal pain, sense of burden, and abnormal lengthening of the ipsilateral scrotum when the patient is in the orthostatic position.

During examination of the patient in standing position, usually the varicocele fills with blood to produce the typical "bag of worms" appearance [2]. It has been reported that many factors can contribute to clinical evolution of varicocele during adolescence, such as sports and different body development.

It is the dilation of the testicular veins of the pampiniform venous plexus that causes a varicocele.

The countercurrent heat exchange mechanism in the spermatic cord vessels is disrupted, which leads to an increased temperature of the testis and scrotum. This may lead to subsequent testicular atrophy and infertility, as first proposed by Tulloch.

Testicular atrophy may be significant in adult life but may become evident quite early in adolescence.

Family History in Varicocele

The association between varicocele and male infertility is strong. This condition is today the main cause of pathologic alterations of the semen. This important association has led to an increased interest of the scientific community toward the epidemiological aspects of varicocele.

The inheritance of varicoceles and their potential transmission to first-degree relatives have been recently investigated. Raman et al. [3] reported in their study that 56.5% of the first-degree relatives of patients with a known varicocele had a palpable varicocele on physical examination. In a prospective study by Mokhtari et al. [4], the authors compared the prevalence of varicocele in first-degree relatives of patients with known varicocele and normal healthy kidney donation volunteers. It was shown that 45.4% of the first-degree family members, especially the brothers (55.1%), had varicocele in the clinical examination.

Another study by Gokçe et al. [5] has demonstrated that the prevalence of varicocele in the first-degree relatives of patients with known varicocele is 33.9%. They found that 36.2% of the brothers of men with varicocele also had an asymptomatic palpable varicocele.

The increased risk of disease occurrence, especially in brothers, is indicative that genetic factors may play an important role in the disease development.

The major genetic and epigenetic factors reported to be associated with varicocele are Y chromosome microdeletions, abnormal methylation DNA, and abnormal frequency or expression of different genes [6].

Some authors [7, 8] also suggest that the Western lifestyle and/or the environmental exposures related to modern industrialization may be a risk factor for the varicocele and for the semen quality, like the presence of endocrine disruptors that can be found in foods and clothes [9].

Family history or incidence of varicocele is rare in those population less affected with varicocele such as black people and Northern Europe Caucasian males.

Definitely, we are sure that in the same family, especially among brothers or among father and son, the body development is similar; it means that somatometric parameters have a fundamental role in the pathogenesis of varicocele.

Relationship Between Varicocele and Somatometric Parameters (Including BMI)

Somatometric parameters are related with varicocele. The most important is the body mass index (BMI). The association between varicocele and BMI is especially evident for adolescents but not for adults [10].

According to somatometric parameters, a greater height is associated with an increased hydrostatic pressure of the spermatic vein, which may strain the valve mechanism in the veins, resulting in the development of varicocele. In

addition, an increased pressure in the left spermatic vein may result from compression of the left renal vein between the aorta and the superior mesenteric artery. This phenomenon is known as the nutcracker effect [11].

Tsao et al. [12] showed that the prevalence and severity of varicocele were inversely correlated with obesity. It indicates that a higher BMI may result in a decreased nutcracker effect because the consequent increased adipose tissue could protect the left renal vein from compression [13].

A controversial element regarding the role of BMI in the development of varicocele is given by the evidence that patients with a higher BMI had a significantly larger left spermatic vein diameter when supine compared to those with lower BMI. This finding does not seem to be correlated with the execution of the Valsalva maneuver. Oppositely with the classical teaching that varicocele is more common in young men who are taller and thinner, this study demonstrates that an increased abdominal pressure while recumbent may contribute to varicocele pathology in the obese population [14].

This represents an important environmental factor, especially for the Western population which is more affected with overweight and obesity.

Relationship Between Varicocele and Those Aspects that Could Affect Clinical Evolution (Sports and Body Development)

The majority of the studies in the scientific literature have shown an important correlation between physical activity, development of varicocele, and consequent semen alterations.

Zampieri et al. [15] demonstrated that the rate of progression to a clinical palpable varicocele in athletes with a subclinical varicocele was greater than that in the normal population; this finding has been confirmed only for patients already affected by spermatic vein reflux (subclinical varicocele). In conclusion, sport practice was only related to an increased probability of developing a palpable clinical varicocele, but it did not correlate with the need for surgery.

In a study of over 1000 young athletes, Rodajevic et al. [16] showed the different role that various sports have in the development of varicocele. They showed that varicocele prevalence was higher in males playing football, basketball, handball, and volleyball; they proposed that these sports necessitated body movements against gravity, which led to the development of varicocele. In the same study, the authors analyzed water polo players and found that the prevalence of varicocele in that group was even lower than the sport-inactive population. The authors showed that after a 6-month period of cessation and abstention from all sporting activity, every parameter of the seminal fluid analysis improved, with a statistically significant improvement in sperm concentration and motility.

Another study has shown that high incidence (up to 30%) of varicocele has been reported in a population of athletes and up to 60–80% in the subgroup of bodybuilders. The incidence of varicocele specifically increases with hours of training, in a linear model [17].

Physical activity is not the only risk factor involved in the pathophysiology of varicocele. In fact, Kumanov et al. [7] speculated that another fundamental element related to the genesis of varicocele is the accelerated growth spurt and pubertal development: the rapid growth could lead to decreased angle of the superior mesenteric artery with the aorta and thus to the nutcracker effect and consequently to a higher hydrostatic pressure in the pampiniform plexus.

Relationship Between Varicocele, Testicular Volume, and Semen Quality in Adolescents

Varicocele is the first cause of male subfertility, and it is well known its correlation with testicular growth arrest. During the past few years, the varicocele was studied in all its etiologic and physiopathological aspects to come to a final conclusion: during childhood, independently from the grade of varicocele, the only objective and valid indication for surgery is the finding of a decreased testicular volume ipsilateral to varicocele. Clinically, it is not yet possible to

determine which patients will develop testicular growth arrest only on the basis of the varicocele grade or the results of the clinical-instrumental tests in order to start early treatment. While different authors reported that it is the type of spermatic vein reflux and not the clinical grade of varicocele that affects the testicular volume and spermatogenesis, it is still controversial the role of semen analysis in adolescents. Recent studies investigate the effects of treatment for varicocele in adolescents to determine their chance of paternity later in life, and the results demonstrated that the presence of varicocele during adolescence did not influence later fertility [18].

Data collected in the literature show as follows: (1) varicocele is a developmental condition clinically related to pubertal development; (2) continuous or intermittent spermatic vein reflux are related with testicular growth arrest, but none is strictly correlated with it; (3) patients with grade 1 varicocele will not develop testicular growth arrest if the clinical grade and the type of spermatic vein reflux remain unchanged over time; (4) patients with grade 2–3 varicocele with normal testicular volume need to be followed with semen analysis; (5) as in adulthood, testicular volume reflects the quality of spermatogenesis, so it is essential to study these patients in order to avoid deteriorations; (6) it is essential to study the semen quality only in Tanner stage V patients, because an improvement in semen parameters during follow-up or after surgery may reflect developmental maturation. In fact, there are still not definitive data about the correlation between age, semen parameters, testicular volume, and Tanner staging [19]; and (7) what is known is that the total testis volume and the testicular volume differences are associated with semen analysis outcome in adolescents, especially for the total motile sperm count [20]. Also, the median semen concentration in patients with testicular asymmetry before surgery is significantly lower compared to those without testicular asymmetry. Therefore, given the existing data, testicular dysfunction in patients with varicocele occurs much earlier than testicular asymmetry, and testicular dysfunction may be detectable before testicular growth arrest [21]. The questionable ethic point is to understand if patients and parents are able to agree for semen test, considering that in many countries, semen analysis can be done only at 18 years old for medicolegal reasons.

Review Criteria

A search of studies was conducted to focus on the epidemiological aspects of varicocele and especially to show the relevant correlation between varicocele and the somatometric parameters of the patient. PubMed, MEDLINE, and Google Scholar were the search engines used.

The search period occurred between April 2018 and May 2018.

The following keywords were used to search for studies and data: "varicocele, adolescents, semen analysis, BMI, familiarity, and sport."

Articles published in languages other than English were not considered. Data that were solely published in conference or meeting proceedings were not included.

Book chapter citations provide conceptual content only.

Multiple Choice Questions and Answers

1. What is the prevalence of different grade of varicocele in peripubertal age?
   (a) **45%, 30%, and 25%, respectively, in grade 1, 2, and 3**
   (b) 40%, 35%, and 25%, respectively, in grade 1, 2, and 3
   (c) 30%, 40%, and 20%, respectively in grade 1, 2, and 3
   (d) 50%, 30%, and 20%, respectively in grade 1, 2, and 3
2. Who is the relative with the highest possibility of being affected with varicocele in a patient with a known varicocele?
   (a) **First-degree relatives, mainly brothers**.
   (b) Only brothers.
   (c) The firstborn.
   (d) There isn't any evidence of familiarity.

3. What is the nutcracker effect? How is it correlated to BMI?
   (a) **It's the compression of the left renal vein between the aorta and the superior mesenteric artery. It is decreased in patients with higher BMI.**
   (b) It's the compression of the left renal vein between the aorta and the inferior mesenteric artery. It is decreased in patients with higher BMI.
   (c) It's the compression of the left renal vein between the aorta and the superior mesenteric artery. It is increased in patients with higher BMI.
   (d) It's the compression of the left spermatic vein between the aorta and the superior mesenteric artery. There is no correlation with BMI.
4. Which statement about the correlation between varicocele and sports is true?
   (a) All of the sport activities worsen the clinical grade of varicocele.
   (b) Sports are not involved at all as risk factors in the genesis of varicocele.
   (c) The cessation of physical activity doesn't interfere with the parameters of the seminal fluid.
   (d) **None of the previous statements is true.**
5. Which statement about the quality of semen in adolescents is true?
   (a) It is always an index of testicular dysfunction.
   (b) It is always related to testosterone levels.
   (c) It has to be done at Tanner stadium 5.
   (d) **All the previous statements are true.**

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Part VII

Varicocele Clinical Practice Guidelines

Adult Varicocele Diagnosis and Treatment

55

Ahmad Majzoub, Chak-Lam Cho, Ashok Agarwal, and Sandro C. Esteves

Key Points

- Varicocele is a clinical diagnosis. Men should be examined in both standing and supine positions. Scrotal ultrasonography may be performed to confirm the presence of varicocele although it may not be recommended in straightforward cases.
- Two semen analyses should be performed in men presenting with varicocele. Advanced sperm function tests such as sperm DNA fragmentation and measures of oxidative stress are valuable, although their routine use is not yet endorsed by international guidelines.
- Varicocele treatment is recommended in infertile men with clinically palpable disease in the presence of abnormal semen analyses and a partner with no identifiable/irreversible female factor of infertility. Varicocele-associated pain represents another indication for varicocele repair.
- Varicocele repair may also be performed in men with low testosterone levels or nonobstructive azoospermia. However, further evidence is required to formulate clear treatment indications in these two scenarios.
- All surgical approaches have a favorable outcome. However, the microsurgical subinguinal approach is associated with the least incidence of varicocele recurrence and postoperative hydrocele formation.

A. Majzoub (✉)
Department of Urology, Hamad Medical Corporation, Weill Cornell Medicine Qatar, Doha, Qatar

C.-L. Cho
S.H. Ho Urology Centre, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

A. Agarwal
American Center for Reproductive Medicine, Department of Urology, Cleveland Clinic, Cleveland, OH, USA

S. C. Esteves
ANDROFERT, Andrology & Human Reproduction Clinic, Campinas, SP, Brazil

Introduction

Varicocele is defined as the abnormal dilatation of the pampiniform plexus of veins within the spermatic cord associated with venous reflux. It is a commonly encountered finding during routine genitourinary examinations as it is prevalent in about 15% of the adult male population [1]. The prevalence was also found to increase with

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age [2], at a rate of 10% each decade, afflicting 75% of men by their eighth decade of life [3]. Within the subfertile population, varicoceles are recognized in 40% of men with primary infertility and in up to 80% of men with secondary infertility [4, 5].

Compelling evidence exists demonstrating a detrimental effect of varicocele on spermatogenesis. Testicular venous stasis, hypoxia [6], and hyperthermia [7] appear to play important roles in varicocele-induced testicular dysfunction although the exact pathophysiology remains not completely understood.

In early times, the surgical ligation of varicocele was performed for painful presentations as it was believed to be a significant cause of orchialgia [8]. This belief is acknowledged in this era as we now know that pain could be a presenting symptom in up to 10% of patients with varicocele [9]. It was not until the famous report by Tulloch in 1952 that the interest in varicocele's impact on male fertility became noticeable [10]. In his report, Tulloch demonstrated the return of sperm to the ejaculate of an azoospermic man after bilateral varicocelectomy who was later able to conceive naturally. Since that time, numerous experimental and clinical studies have been conducted exploring either the pathophysiology of varicocele or the outcomes of its treatment on male fertility. Controversies emerged and were principally based on the fact that despite its observed detrimental effects on testicular function, a good number of men with varicocele are still able to conceive naturally. This triggered scientific societies such as the American Urological Association (AUA) [11], American Society for Reproductive Medicine (ASRM) [12], and European Association of Urology (EAU) [13] to publish clinical guidelines that would aid in selecting treatment candidates and thereby recognizing the indications for varicocele repair. This chapter aims to review the methods for varicocele diagnosis and treatment highlighting the recommendations set forth by international guidelines in an evidence-based approach.

Diagnosis

Physical Examination

Varicocele is principally a clinical diagnosis. While some men do present with scrotal pain or swelling, the condition is asymptomatic in the majority of cases. Varicocele pain is more commonly reported by men who engage in strenuous activities. It is described as a heavy dragging sensation or sometimes hotness that is felt after prolonged standing or physical activity and which is relieved by rest.

Physical examination should be initiated while the patient is in the standing position starting with inspection of the scrotum. Apparent venous distention around the spermatic cord, which usually resemble a bag of worms, indicates the presence of grade 3 varicocele. The spermatic cord is then gently palpated. Thickening of the spermatic cord, which is usually felt as a squishy tube, indicates the presence of grade 2 varicocele. On the other hand, a grade 1 varicocele is considered only when a filling sensation is felt between the fingers of the examiner as the patient performs the Valsalva maneuver [14].

The patient should then be examined in the supine position, during which a palpable idiopathic varicocele should disappear. However, if swelling persists, especially on the right side, it should raise suspicion for the presence of secondary causes of varicocele such as retroperitoneal tumors, or lymphadenopathy.

Testicular size assessment is an important step of the examination as the varicocele can halt testicular growth. In expert hands, testicular size speculations are fairly accurate. However, caliper measurements of testicular dimensions can also be performed. A variety of devices for testicular volume assessment can also be utilized including the Prader orchidometer [15] and the Rochester (Takihara) orchidometer [16] (Fig. 55.1).

Laboratory Investigations

Conventional semen analysis should be performed for patients presenting with infertility or

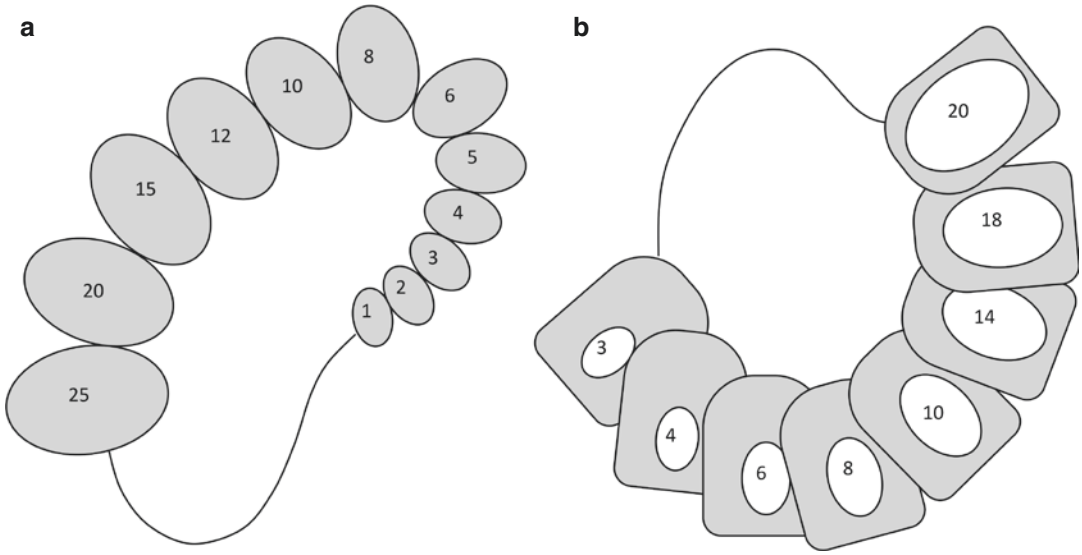

Fig. 55.1 Diagram representing the appearance of (**a**) Prader orchidometer (a string of ovoid-shaped beads of increasing sizes) and (**b**) Rochester orchidometer (a ring of cards with open spaces to accommodate the shape of a testis)

as a baseline for symptomatic patients before varicocele treatment for medicolegal purposes. Sufficient evidence exists linking varicocele to altered semen parameters including sperm concentration, total and progressive motility, and normal morphology [17]. Nonetheless, about 12% of infertile men with varicocele may have a normal conventional semen analysis result [18]. This finding has triggered researchers to investigate specialized sperm function tests during the evaluation of varicocele patients to help in treatment decision-making. Measures of oxidative stress (OS) and sperm DNA fragmentation (SDF) are most commonly utilized in this regard [19]. Varicocele through various pathophisiologic mechanisms such as scrotal temperature elevation, testicular hypoxia, and necrosis is believed to induce a state of OS that would ultimately aggravate SDF, sperm membrane lipid peroxidation, and abortive apoptosis [20]. While current guidelines do not recommend the routine use of these tests in the evaluation of varicocele patients, numerous studies confirmed their contribution to infertility in this patient population [19, 21–23]. Higher levels of OS products such as reactive oxygen species, nitric oxide, and lipid peroxidation markers have been observed in infertile varicocele patients compared with infertile patients without varicocele [24, 25]. Moreover, men with varicocele, regardless of their fertility status, were found to have higher SDF levels than controls, indicating a direct relationship between varicocele and sperm DNA damage [25, 26].

Serum hormone evaluation has recently gained attention based on reports revealing the presence of low testosterone levels in varicocele patients secondary to some degree of Leydig cell dysfunction. As serum testosterone levels are influenced by the circadian rhythm, early morning samples should preferably be measured. Tanrikut et al. compared serum testosterone levels of 325 varicocele patients with 512 vasectomy reversal patients [27]. The authors observed significantly lower testosterone levels in varicocele patients of various age groups in comparison to the control group. The negative impact that varicocele has on Leydig cell function has also been confirmed in animal studies with surgically induced varicocele [28, 29]. Luo et al. reported significant reductions in intratesticular testosterone in congruence with elevation of apoptosis index of Leydig cells 4–8 weeks following experimental varicocele induction in rats [28].

Further evidence supporting the association between varicocele and low testosterone levels came from post-varicocelectomy studies, which revealed significant improvements in testosterone levels following treatment [30]. While all the society guidelines did not include hormone evaluation in the workup of varicocele patients, the ASRM acknowledged that there is increasing evidence linking varicocele with impaired testosterone production, pointing out that some clinicians may advocate varicocele repair in the setting of decreased testosterone levels [12].

Imaging Studies

There is controversy regarding the routine use of scrotal Doppler scanning in patients with varicocele between different guidelines. While the AUA and the ASRM do not recommend its utility [11, 12], the EAU favors its use as a confirmatory study in varicocele patients [13]. Many clinicians elect to perform scrotal Doppler scanning when the physical examination is difficult (obese patient) or suspicious for testicular lesions or when there is a history of prior scrotal surgery or suspicion of varicocele recurrence.

Scrotal ultrasound is a very sensitive (97%) and specific (94%) tool for the diagnosis of varicocele [31]. It accurately assesses testicular volume, venous diameter, and reversal of venous flow with the use of duplex-enhanced imaging. A venous diameter of 3 mm is generally accepted as a cutoff value for diagnosing varicocele [32]. An ultrasound scoring system for varicocele diagnosis has been proposed by Chiou and colleagues. It incorporates maximal venous dilatation (score 0–3), the presence of a venous plexus, the sum of the diameters of veins in the plexus (score 0–3), and the change of flow on Valsalva maneuver (score 0–3). A total score of 4 or more was used to define the presence of a varicocele [33].

Other imaging studies such as thermography, radionuclide scanning, and spermatic venography are not routinely used for the diagnosis of varicocele. Only venography may have a role in patients undergoing an embolization procedure or in the evaluation of varicocele recurrence [12].

Treatment

Indications for Treatment of a Varicocele

Varicocele is the most common correctable cause of male infertility. However, selecting the appropriate varicocele patient who would benefit from treatment has been subject to considerable debate over the past few decades. This controversy stems from the findings of several retrospective studies reporting conflicting results on the benefit of repair on semen parameters or fecundity. Moreover, many of these reports have been criticized for their small sample size, discrepancy in varicocele definition and detection, varied treatment modality, and most importantly inclusion of subclinical varicoceles in their study groups.

Recently, meta-analyses of randomized controlled trials (RCT) revealed meaningful benefits following varicocele treatment provided that correct indications were met. Baazeem et al. [34] reviewed four RCTs reporting significant improvement in sperm concentration [+12.32 million/ml (95% CI, 9.45–15.19; $p < 0.0001$)] and total motility [+10.86% (95% CI, 7.07–14.65; $p < 0.0001$)] following varicocele ligation. With regard to the effects of varicocele ligation on natural pregnancy rate, a significant benefit was only obtained when surgery was performed on patients with clinical varicocele and abnormal semen analysis results. Kim et al. [35] conducted a meta-analysis on 7 RCTs including 610 infertile men, some with subclinical varicocele and normal semen analysis. Their initial assessment revealed an insignificant benefit from varicocelectomy with an odds ratio (OR) of 1.90 ($p = 0.162$). However, their sub-analysis of patients with clinical varicocele and impaired semen quality significantly favored varicocelectomy with an OR of 4.15 ($p < 0.001$) for natural pregnancy. Similar findings were detected in the most recent Cochrane review where the initial analysis of 10 RCTs including men with subclinical varicocele and normal semen parameters failed to detect a significant improvement in natural pregnancy rate following varicocelectomy [36]. However, the subgroup analysis of patients

with clinical varicoceles and abnormal semen parameters favored varicocelectomy with an OR of 2.39 ($p = 0.03$).

As such, the ASRM Practice Committee report [12] and the AUA best practice policy [11] on the indications for varicocele repair echo these findings recommending treatment if the following conditions are met: " [1] the varicocele is palpable on physical examination of the scrotum; [2] the couple has known infertility; [3] the female partner has normal fertility or a potentially treatable cause of infertility, and time to conception is not a concern; and [4] the male partner has abnormal semen parameters."

Other indications proposed by the ASRM and the AUA include symptomatic varicocele and clinically palpable disease with abnormal semen analysis in a man who is not necessarily seeking fertility at the moment of his presentation [11, 12].

The EAU guidelines [37], on the other hand, recommend varicocele repair in patients with clinically palpable disease, oligozoospermia, and otherwise unexplained infertility in the couple. However, they have not addressed the female partner fertility status.

The ASRM contraindications to treatment include subclinical varicocele, normal semen parameters, and/or isolated teratozoospermia[12]. Current evidence does not support male infertility treatment for isolated abnormal sperm morphology as its effects on reproduction are still not clearly understood [38–40]. In fact, the AUA Best Practice Statement on the Optimal Evaluation of the Infertile Male recommends that therapeutic decisions should not be based on abnormal strict morphology when not accompanied by other semen parameter abnormalities [41].

The EAU guidelines, on the other hand, dismisses varicocele treatment in men with subclinical disease and/or normal semen parameters without specifically addressing isolated teratozoospermia [13].

Varicocele repair has been also considered in patients with high levels of sperm DNA fragmentation. A recent guideline endorsed by the society for translational medicine, identifying the indications for sperm DNA fragmentation testing in clinical practice, has recommended its use in patients with high-grade varicocele with normal conventional semen parameters and low-grade varicocele with borderline/abnormal conventional semen parameters suggesting that this test can aid in treatment decision-making [42].

Treatment Options

Varicocele can be treated with surgical ligation or radiographic embolization. Several surgical approaches exist and are classified according to the technique (conventional, microsurgical, or laparoscopic) or the level of testicular vein ligation (high or low) or whether the gonadal artery is spared or ligated. In recent years, varicocele surgery has been influenced by advancements in optical magnification and microsurgery which is favored by an increasing number of urologists. This is clearly obvious by the results of physician surveys which revealed that in comparison to only 6% of urologists performing microsurgical varicocelecomy in 1994 [43], 48% are utilizing loop magnification and 16% microsurgery in 2008 [44].

Controversy once existed with regard to testicular artery sparing during the course of varicocelectomy. The nonartery-sparing approach is favored by some clinicians who have concerns regarding varicocele recurrence which may occur if the *venae comitantes* coursing along the testicular artery are missed [45]. However, with the use of microsurgery in addition to intraoperative Doppler probes, artery-sparing varicocelectomy can now be efficiently achieved (Fig. 55.2). Furthermore, artery ligation could have a detrimental effect on testicular function evidenced by seminiferous tubular atrophy that has been reported following this maneuver [46].

Conventional Varicocelectomy

The retroperitoneal approach was described by Palomo in 1949 [47]. It involves a muscle-splitting incision performed at the level of the internal inguinal ring. Testicular vessels are then identified and ligated at the place of least

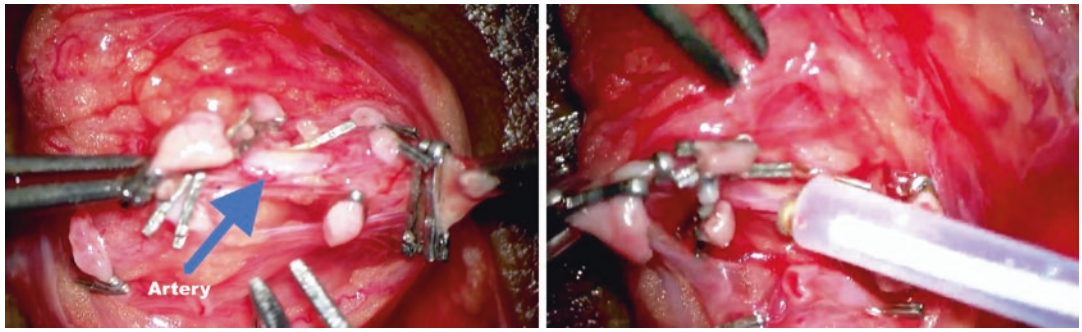

Fig. 55.2 Microsurgical subinguinal varicocelectomy. The artery is spared with proper magnification and use of a Doppler probe

vascular branching. Artery sparing has been performed with the Palomo technique. However, it was associated with a high incidence of postoperative recurrence [48].

The inguinal approach, described by Ivanissevich [49] in 1960, is carried out with an incision that is made over the inguinal canal. It requires opening of the external oblique aponeurosis in order to deliver the spermatic cord. The exposed cord is then explored to identify and ligate its spermatic veins. Artery-sparing and nonartery-sparing variations can be easily performed with the inguinal approach. While an effective technique, it has the disadvantage of requiring the patient to have delayed return to full activity due to the extensive muscular dissection in the inguinal canal.

The subinguinal approach, described by Marmar in 1985 [50], is performed through an incision at or near the pubic tubercle avoiding the need to open the external oblique aponeurosis. Advantages of this technique include less pain, smoother recovery, and easier access to the spermatic cord especially among obese patients or those with previous inguinal surgery. However, a greater number of veins are present at such a low level, making the procedure technically challenging.

Microsurgical Varicocelectomy

Microsurgery was integrated into varicocelectomy soon after its introduction into the field of urology in the 1970s [51]. The main objective for using microsurgery in varicocele ligation was to reduce the incidence of varicocele recurrence and hydrocele formation. Meticulous vein ligation and sparing of lymphatics can certainly be achieved with greater accuracy with adequate magnification. This was confirmed by reports from Minevich and Goldstein who demonstrated significantly lower recurrence rates and hydrocele formation after microsurgical varicocelectomy [52, 53]. These superior surgical outcomes have made microsurgical varicocelectomy the gold standard technique for varicocele repair [54].

Laparoscopic Varicocelectomy

Laparoscopic varicocelectomy was first performed by Hagood in 1992 [55] and entails the use of hemostatic clips or bipolar coagulation to obliterate the spermatic veins [56].

While it is considered an acceptable approach for varicocele ligation, its inherent drawbacks include the need for pneumoperitoneum, prolonged operative time, and higher cost [43]. Furthermore, complications unique to this approach, such as intestinal or major vascular injury, pneumothorax, and incisional hernia while rare, can be disastrous.

The laparoscopic approach was also criticized by its higher incidence of postoperative varicocele recurrence and hydrocele formation in comparison to the subinguinal microsurgical approach. Few authors have thus tried lymphatic-sparing techniques in attempts to decrease the incidence of postoperative hydrocele. Rizkala et al. were

able to reduce the postoperative hydrocele rate to 4.5% with the lymphatic-sparing technique in comparison to 43% with the standard laparoscopic surgery [57]. Advancements in the field of laparoscopy have also been endeavored in varicocele surgery. Varicocelectomy has been performed via natural orifice transluminal endoscopic surgical approach (NOTES) [58] and laparoendoscopic single-site (LESS) surgery [59] with promising results. However, such techniques require validation by large, randomized, clinical trials with endpoints such as patient safety, surgical outcome, and procedure cost taken into account.

Angiographic Embolization

Antegrade or retrograde embolization or sclerotherapy is carried out by the interventional radiologist who usually accesses the venous system through the groin. After confirming their position within the testicular vein via angiography, coils or sclerosing agents are applied to obliterate venous flow. Despite the minimally invasive nature of such a procedure, technical challenges can be encountered resulting in a failure rate of up to 20% of cases due to aberrant venous anatomy, difficulty in cannulating the testicular vein, and extravasation of contrast during the procedure [60, 61]. High varicocele recurrence is another important drawback that has disfavored urologists from recommending angiographic embolization as the primary treatment method for varicocele and to consider it as an alternative option in patients with documented varicocele recurrence after surgical ligation.

The EAU guidelines have recognized microsurgical varicocelectomy as the most effective treatment approach [13]. Their finding was based on the lower incidence of recurrence and complication rates with microsurgical varicocelectomy compared with the other approaches. While the ASRM and the AUA guidelines have in fact echoed the same outcomes, they did not recommend a particular treatment approach over the other noting that "the treating physician's experience and expertise, together with the options available, should determine the choice of varicocele treatment." [11, 12]

Results of Treatment

Few meta-analyses exist comparing fertility outcome and complication rates between different surgical approaches. Ding et al. [62] evaluated 4 RCTs including 1015 patients reporting significantly higher pregnancy rates following microsurgical varicocelectomy compared with open varicoceletomy only (OR 1.63, $p = 0.002$). However, the authors observed significantly lower varicocele recurrence and hydrocele formation rates following microsurgical varicocelectomy compared with open (OR 0.13, $p < 0.001$; OR 0.09, $p < 0.001$, respectively) and laparoscopic varicoceletomy (OR 0.12, $p < 0.001$; OR 0.05, $p = 0.003$, respectively). Another meta-analysis by Cayan et al. [63] included 36 studies, and 4473 varicocele patients revealed a natural pregnancy rate of 41.97% following microsurgical varicocelectomy which was significantly higher than the ones reported by retroperitoneal (37.69%), laparoscopic (30.1%), macroscopic inguinal (36.0%), and radiographic embolization (33.2%) approaches. The authors also noted a significantly lower varicocele recurrence rate with microsurgical varicocelectomy (1.05%) compared with the retroperitoneal (14.97%), the laparoscopic (4.3%), the macroscopic inguinal (2.63%), and the radiographic embolization (12.7%) approaches. Postoperative hydrocele formation was also significantly lower in the microsurgical approach (0.44%) compared with the retroperitoneal, laparoscopic, and macrosurgical inguinal approaches (8.24%, 2.84%, and 7.3%, respectively). The ASRM, AUA, and EAU guidelines have compared the incidence of hydrocele formation between the different treatment approaches (Table 55.1).

Varicocele recurrence in high-ligation approaches has been attributed to the higher likelihood of missing venous ramifications of the external spermatic vein which were proven to be dilated in up to 75% of cases [64]. This obstacle is easily overcome in the low-ligation approaches which are also considered ideal in identifying and preserving lymphatics and reducing the risk of postoperative hydrocele.

Table 55.1 Comparison between present clinical guidelines on varicocele management

	AUA/ASRM	EAU
Evaluation	Medical and reproductive history, a physical examination, and at least two semen analyses	Medical history and physical examination and semen analysis. One test is sufficient if normal
Physical examination	Should be performed in both the recumbent and upright positions Three clinical grades identified	Three clinical grades identified
Scrotal ultrasonography	Only indicated in an inconclusive physical examination of the scrotum	Should be routinely performed to confirm varicocele presence
Other ancillary radiologic tests	Spermatic venography may be performed in varicocele recurrence or persistence after varicocele repair	X-ray can be useful if antegrade or retrograde sclerotherapy or embolization is planned
Indications for treatment	Palpable varicocele AND infertile couple AND abnormal semen parameters or abnormal results from sperm function tests AND normal or potentially treatable female cause of infertility	Treat varicoceles in men with a clinical varicocele, oligospermia, and otherwise unexplained infertility in the couple
Additional indications	Palpable varicocele + abnormal semen parameters in a man not seeking fertility Increasing evidence that larger varicoceles may impact testosterone production and some advocate repair in the setting of diminished testosterone levels[a]	None
Treatment approach	The treating physician's experience and expertise, together with the options available, should determine the choice of varicocele treatment	Current evidence indicates that microsurgical varicocelectomy is the most effective method among the different varicocelectomy techniques
Follow-up	Semen analysis at 3-month intervals for at least 1 year or until pregnancy occurs	None
Treatment of recurrence/ persistence	Surgical ligation or embolization	None
Recurrence rates	Retroperitoneal and laparoscopic: Up to 15% Low inguinal/subinguinal: 1–2% Embolization: 15%	Antegrade sclerotherapy: 9% Retrograde sclerotherapy: 9.8% Retrograde embolization Inguinal approach: 13.3% High ligation: 29% Microsurgical inguinal or subinginal: 0.8–4% Laparoscopic: 3–7%
Varicocele and ART	ART may be considered the primary treatment option when there is an independent need for such techniques to treat a female factor, regardless of the presence of varicocele and suboptimal semen quality In certain circumstances, treatment of a varicocele should be considered before ART even when a significant female factor is present. Specifically, men with NOA have been shown to respond to varicocele repair, albeit in fairly low-quality observational studies[a]	None

AUA American urological association (report on varicocele and male infertility), *ASRM* American Society for Reproductive Medicine (report on varicocele and infertility: a committee opinion), *EAU* European Association of Urology (guidelines on male infertility), *ART* assisted reproductive therapy, *NOA* nonobstructive azoospermia; [a]statement endorsed only by the ASRM

The AUA and ASRM guidelines have been presented together as the ASRM 2015 report can be considered as an update to the previous joint report by AUA/ASRM

The degree of magnification used in the low-ligation approaches can also have a significant impact on postoperative complication rates. Cayan et al. studied 100 patients undergoing inguinal and subinguinal varicocelectomy revealing a 0% recurrence rate with microsurgery, 2.9% with loupe magnification, and 8.8% without magnification [65]. The authors also reported similar postoperative hydrocele outcomes with the methods of magnification used (0%, 2.9%, and 5.9%, respectively).

The operating surgeon's level of training/experience along with the operative times has long been considered as the main drawbacks to microsurgical varicocelectomy. However, these two factors appear to be inversely related to each other. Watanabe et al. [66] compared the operative times between retroperitoneal, laparoscopic, and microsurgical subinguinal approaches performed on 144 varicocele patients. The authors revealed that in fact, a significantly lower operative time was detected with the microsurgical approach (86.3 ± 28.4 minutes) compared with the retroperitoneal (111.8 ± 21.1 minutes) and the laparoscopic (109.4 ± 27.3 minutes) approaches ($p < 0.01$). This finding suggests that in high-caseload centers and in the hands of urologists with microsurgical training, microsurgical subinguinal varicocelectomy appears to be the quickest, safest, and most effective procedure for varicocele repair.

Follow-Up Evaluation

Patients who underwent varicocele repair should be followed with physical examination to rule out varicocele persistence or recurrence. If such a condition is identified, we believe that scrotal ultrasonography is the most appropriate next step to document the presence of venous reflux. However, the ASRM and AUA suggest performance of internal spermatic venography followed by either surgical ligation or percutaneous embolization of the refluxing veins [11, 12]. This is principally because these societies favor radiographic treatment in recurring varicoceles proposing venography for diagnosis with the potential of therapeutic intervention at the same setting. Most studies assessing the effectiveness of treatment of varicocele recurrence have employed the microsurgical subinguinal or angiographic embolization approaches [67–69]. While no head-to-head comparisons are available, both methods can be performed in this patient population. We recommend microsurgical subinguinal varicocelectomy in patients who had initially undergone a high-ligation procedure and radiographic embolization for patients who had undergone a low-ligation procedure. This is because a redo subinguinal varicocelectomy, while not impossible, could be technically challenging [70].

Repeat semen analysis should be performed every 3 months during the first year or until pregnancy is achieved. While Al Bakri et al. [71] advocated that improvements in semen parameters are only expected at 3 months' post varicocelectomy indicating that no further improvement should be expected afterward; others have proven this assumption to be not entirely true revealing that further improvements could be obtained 6 months following surgery [72, 73]. Pregnancy is mainly related to the degree of improvement in semen parameters irrespective of the time it takes for such an improvement to occur [72].

Treatment in the Era of Assisted Reproduction

In the current era, the major advancements witnessed in the field of assisted reproduction have widened its indications, especially among gynecologist, as a means to overcome various male factors. Intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI) are commonly used in the setting of varicocele to bypass the semen abnormality and achieve pregnancy. While this is possible, few issues arise such as the impact of varicocele on ART outcome and the increased cost of these procedures. As stated previously, significant associations between varicocele and raised SDF levels exist. The implications of high SDF on the outcomes of ART have been recently described

and include increased risk of IUI and IVF failure and increased risk of recurrent abortions following ICSI [26]. Since varicocelecomy is proven to reduce SDF values, it appears reasonable to treat the varicocele prior to undergoing ART. Furthermore, the improvements in semen parameters seen following varicocele repair may allow a couple with severely impaired semen parameters to have less invasive ART treatments—a statement that was acknowledged by the ASRM guidelines [12]. On the other hand, cost-effectiveness analyses comparing varicocele repair to other ARTs have been published previously. Schlegel calculated the direct and indirect costs of varicocelectomy and IVF/ICSI and reported significantly lower costs per live birth for varicocele repair ($26,268) compared with IVF/ICSI ($89,091) [74]. Pearson et al. [75] compared the cost-effectiveness of four treatment plans that can be implemented in a varicocele scenario. The treatments were (i) observation, (ii) varicocelectomy followed by up to three IVF cycles if no pregnancy was achieved 1 year after surgery, (iii) three IUI cycles followed by three IVF cycles if the IUI failed, and (iv) up to three immediate IVF cycles. Although indirect costs were not calculated in this analysis, the authors revealed that varicocelectomy/IVF was the most beneficial and cost-effective approach, contrary to immediate IVF that was the least beneficial and most expensive method. The probability of live birth following varicocelectomy/IVF in this report was 72% with an average cost/delivery of $32,171, while that of immediate IVF was 61% with an average cost of $39,001. Factors such as the female age and fertility status and the time available for conception can be considered to help in treatment decision-making.

Another important circumstance is when a varicocele is diagnosed in a patient with nonobstructive azoospermia. Reports have indicated that varicocele repair may allow sperm to return to the ejaculate in 10–55% of patients with testicular histology showing hypospermatogenesis and maturation arrest [76–78]. While the histology result may not be available at the time of decision-making, it is important to counsel the couple about the predictors of improvement in semen parameters following surgery and the likelihood of success in this scenario [79]. The ASRM has noted that varicocelectomy may be considered in appropriately selected patients as the anticipated improvement in semen status may preclude the need for sperm retrieval procedures before or during an IVF/ICSI cycle [12].

Conclusion

Varicocele is a common cause of impaired semen parameters and male factor infertility. Its diagnosis is made with a proper physical examination and can be confirmed with scrotal ultrasonography. Surgery is indicated in infertile patients with clinically palpable disease and abnormal semen parameters together with normal/potentially treatable female factors for infertility. Among the different surgical approaches, microsurgical subinguinal varicocelectomy is the most favored as it is associated with the best reproductive outcome and the least incidence of postoperative complications.

In face of the increasing number of varicocele-related research, guidelines and Practice Committee reports on varicocele management will certainly have more to address in the future. Areas that await expert recommendations include (i) development of predictive models that can aid in patient selection for varicocele repair; (ii) utility of specialized sperm function tests, namely, SDF and OS measures, in the initial workup of varicocele patients; and (ii) clear standpoints on the indications of surgery in azoospermic varicocele patients.

Review Criteria

An extensive search of the literature was done using scientific search engines including PubMed, MEDLINE, ScienceDirect, and Google Scholar. Search criteria included the following keywords: “varicocele,” “diagnosis,” “treatment,” “guidelines,” “varicocele recurrence,” and “hydrocele.” Data from published papers or book chapters were included.

Multiple Choice Questions and Answers

1. The initial evaluation of male varicocele may include all except:
   (a) Semen analysis
   (b) Medical and reproductive history
   (c) Physical examination
   (d) **Infrared digital thermography**
   (e) Scrotal ultrasonography
2. Varicocelectomy:
   (a) Is indicated in patients with subclinical disease
   (b) Is not indicated in the current era due to advancements in ART
   (c) Is not an option in men with testicular pain
   (d) **May be performed in men with low serum testosterone levels**
   (e) None of the above.
3. Regarding the surgical approach that can be used in varicocele repair.
   (a) Not a single approach has been favored by all clinical guidelines including AUA, ASRM, and EAU
   (b) Laparoscopic varicocelectomy is favored in varicocele recurrence
   (c) **Microsurgical subinguinal varicocelectomy appears to be the best approach as it is associated with lower risk of varicocele recurrence and hydrocele formation**
   (d) Nonsurgical, less invasive approaches are preferably performed
   (e) None of the above.
4. Varicocele recurrence is least common in
   (a) **Microsurgical varicocelectomy**
   (b) Laparoscopic varicocelectomy
   (c) Open varicocelectomy
   (d) Radiographic embolization
   (e) Radiographic sclerotherapy
5. Varicocele repair in the setting of ART.
   (a) Should always be performed initially
   (b) Should not be performed at all
   (c) **May be indicated in patients with non-obstructive azoospermia**
   (d) Can be performed in a patient with subclinical varicocele if the female partner is of younger age
   (e) Should be performed in a patient with clinical varicocele if the female partner is of older age

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Pediatric and Adolescent Varicocele Diagnosis and Treatment

Matheus Roque and Sandro C. Esteves

Key Points

- A varicocele is found in 11–16% of postpubertal boys.
- Approximately 20% of adolescents with a varicocele will face difficulties in fathering a child later in life.
- The majority of adolescent varicoceles are asymptomatic.
- In adolescents, a testicle is considered hypoplastic if there is an asymmetry of at least 20% or if it is 2 mL smaller compared to the contralateral testicle.
- The American Urological Association (AUA), American Society for Reproductive Medicine (ASRM), European Association of Urology (EAU), and European Society of Pediatric Urology (ESPU) have issued guidelines with a primary objective to offer pediatric urologists and other healthcare professionals the best evidence for the diagnosis and management of the children and adolescents with varicocele.
- There are significant differences in the methods of guidelines' development, data collection, and analysis. Furthermore, there are differences between the European and American approaches to varicocele diagnosis and management in adolescents. However, the existing guidelines concur that treatment should be offered when there is a palpable varicocele with evidence of testicular hypotrophy.

Introduction

Varicocele is as an abnormal dilatation of testicular veins in the pampiniform plexus associated with venous reflux [1]. Varicocele can negatively impact the male reproductive potential, and it is also related to disrupted endocrine function, in particular testosterone production [2, 3]. Although varicocele typically emerges during adolescence, it is usually not diagnosed until later in life [1]. Varicocele is one of the most common genital conditions referred to pediatric urologists [4, 5]. Although varicocele is rarely seen in children, in whom the prevalence is lower than 1% [6], it is present in 11–16% of postpubertal (12–18 years) boys [7, 8].

M. Roque (✉)
Department of Reproductive Medicine, ORIGEN – Center for Reproductive Medicine, Rio de Janeiro, Brazil
e-mail: matheusroque@origen.com.br

S. C. Esteves
ANDROFERT, Andrology & Human Reproduction Clinic, Campinas, SP, Brazil

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The majority of adolescent varicoceles are asymptomatic. However, a varicocele diagnosed during adolescence raises concerns about the future fertility as it has been associated with hypotrophy/atrophy of the testis, modifications in seminal parameters, and male infertility. It has been shown that fertility problems will arise later in life in about 20% of adolescents with varicocele [5, 9, 10]. Indeed, varicoceles are found in about 25% of the men presenting for an infertility workup [1]. Thus, early intervention would be desirable to avoid disease progression [5, 9, 10]. Nevertheless, there is a relative paucity of studies investigating varicocele in adolescents, making it difficult not only to establish for whom treatment would be beneficial but also to decide when to initiate and which type of therapy fits better this specific population [11, 12].

Although management of varicocele in adolescent has evolved over the last 30 years, it remains a controversial topic in pediatric urology [12]. The ability to precisely determine the effect of varicocele in future fertility is challenging, due to limitations in obtaining and interpreting semen analysis in children and adolescents. Moreover, testicular asymmetry, which is a critical parameter in children/adolescents with varicocele, can be related to differential testicular growth during puberty regardless of varicocele presence [13]. Thus, current research focuses on identifying adolescents more likely to benefit from interventional therapy. Along these lines, Clinical Practice Guidelines (CPG) and Best Practice Statements (BPS) have been developed aiming at improving efficiency, enhancing research opportunities, and creating a cost-effective diagnosis and treatments strategies [14].

Currently, there are four guidelines endorsed by a provider organization concerning the diagnosis and management of varicocele in children and adolescents. These guidelines were issued by the American Urological Association (AUA) [15], American Society for Reproductive Medicine (ASRM) [16], European Association of Urology (EAU) [17], and European Society of Pediatric Urology (ESPU) [18].

These CPG and BPS aim to offer pediatric urologists and other healthcare professionals the best evidence for diagnosis and management of children and adolescents with varicocele, ultimately enhancing the quality of healthcare and avoiding potentially harmful or ineffective interventions during evaluation and management. In this chapter, we summarize the contents of CPG and BPS of pediatric and adolescent varicocele. Furthermore, we provide a critical assessment of their methods and recommendations.

Diagnosis

The varicocele diagnosis is based on history and physical examination. The latter represents the gold standard for diagnosing clinically relevant varicoceles and should be carried out in a warm environment with the patient in standing and supine positions. In the standing position, the patient must be instructed to perform the Valsalva maneuver to grade the small varicoceles adequately. The grading scale developed by Dubin and Amelar is the most commonly used assessment tool, which categorizes varicoceles from grade I through III [19]. Grade I varicoceles are palpable only with Valsalva. Grade II varicoceles are visible with Valsalva pressure and palpable without Valsalva. Grade III varicoceles are visible without Valsalva and are traditionally referred to have a "bag of worms" appearance [20, 21]. An important aspect to be considered in pediatric and adolescent population is testicular volume asymmetry. This essential aspect should be evaluated during a physical examination, with the use of an orchidometer or by ultrasound. An ultrasound performed with Doppler presents high sensitivity and specificity, mainly in the pediatric population [21], and is often used for active surveillance of varicocele impact on testicular growth [22].

Notwithstanding, estimating testicular volume with the use of an orchidometer is a valid alternative [23]. Although specific evidence-based criteria for ultrasonography diagnosis of varicocele are lacking, the current consensus indicates that multiple spermatic veins >2.5–3.0 mm in diameter (at rest and with Valsalva) tend to correlate with the presence of clinically significant varicoceles

[19]. The semen analysis is not widely utilized in the pediatric and adolescent varicocele patients because an abnormal result might be related to an immature hypothalamus-hypophysis-gonadal axis rather than the varicocele. A recently published survey with pediatric urologists has shown that only 13% of the doctors routinely use the seminal analysis from their patients in their practice [21].

AUA and ASRM Guidelines

These guidelines recommend physical examination to be performed with the patient in both recumbent and erect position. It is suggested that a palpable varicocele can be detected in the erect position but disappears or significantly diminishes when the patient is in the supine position. Both guidelines suggest that varicoceles should be graded according to the Dubin and Amelar classification, ranging from grade I to III. When a suspected varicocele is not palpable, the scrotum should be examined in a standing position under a Valsalva maneuver. Concerning ultrasonography, the AUA guideline suggests that it should be only performed for clarification, in cases of an inconclusive physical examination of the scrotum. There are no comments concerning the testicular asymmetry.

EAU Guideline

Although this guideline recommends that the initial diagnosis of a varicocele be made by clinical examination in the upright position, unlike the guidelines discussed above, the EAU guideline recommends that the ultrasound with color duplex analysis should be performed to confirm the diagnosis made by physical examination.

ESPU Guideline

This guideline states that the diagnosis should be made by physical examination with the patient in an upright position. This is the only guideline that established recommendations concerning testicular asymmetry. It is suggested that the size of both testicles should be evaluated by an orchidometer or ultrasound to determine whether or not testicular hypotrophy exists. A testicle is considered hypoplastic if it is smaller than 2 mL or if there is an asymmetry of at least 20% compared to the contralateral testicle. In the ESPU guidelines, there is also a recommendation to perform renal ultrasound examination in prepubertal boys and patients with isolated right varicocele because an extension of Wilms' tumor into the renal vein and inferior vena cava might be associated with a secondary varicocele.

Treatment Indications

AUA and ASRM Guideline

Notably, despite not utilizing testicular hypotrophy for diagnosis, these guidelines recommend that the indication for treatment should be based on the presence of a unilateral or bilateral varicocele that is associated with testicular hypotrophy as catch-up growth has been demonstrated. Moreover, they also suggest that treatment should be offered when semen abnormalities are detected as the reversal of semen abnormalities has been observed. However, these guidelines acknowledge that data are lacking regarding the impact of treatment on future fertility. Annual follow-up with an objective measurement of testis size and/or semen analyses to monitor for earliest sign of varicocele-related testicular injury is recommended if there is no decrease in testicular size initially.

EAU Guideline

The EAU guidelines recommend varicocele treatment to adolescents with progressive failure of testicular development documented by serial clinical examination (grade B recommendation). However, there are concerns that there is a significant risk of overtreatment of varicoceles in adolescents (level of evidence 3).

ESPU Guideline

The ESPU guidelines indicate that the criteria for varicocelectomy in children and adolescents are as follows:

- Varicocele associated with a small testis;
- An additional testicular condition affecting fertility;
- Bilateral palpable varicocele;
- Abnormal sperm quality (in older adolescents);
- Symptomatic varicocele.

Testicular hypoplasia is defined as a testis that is smaller by >2 mL or 20% compared to the other testis. Repair of a large varicocele, physically or psychologically causing discomfort, may also be considered.

Treatment Method

AUA and ASRM Guideline

The ASRM and AUA guidelines suggest that both surgery and percutaneous embolization may be performed, although they acknowledge that the recurrence rate can be different when comparing both techniques. The surgical intervention can be performed by open retroperitoneal, inguinal, subinguinal, or laparoscopic approaches. Percutaneous intervention can be accomplished by percutaneous embolization of the refluxing internal spermatic vein(s).

EAU and ESPU Guideline

Both guidelines suggest that the best treatment method is the one that the doctor is most familiar with. However, they recognize that the microsurgical approach is both more effective and associated with less morbidity when comparing to other methods. The ESPU guideline favors surgical ligation and recommends the use of some form of magnification, such as microscopic or laparoscopic (level of evidence 2; grade B recommendation). Concerns about the cost-effectiveness of the laparoscopic approach have been raised.

Guidelines' Critical Evaluation

The existing guidelines are informative with a real chance to influence the readers positively. However, there is a lack of information about the cost-effectiveness and risk-benefit analysis of the techniques employed to treat the patients concerned. Moreover, the guidelines issued by AUA and ASRM do not provide evidence-based levels for the recommendations given. When evaluating the EAU and ESPU guidelines, most of the recommendations derive from nonrandomized clinical trials, retrospective studies, and expert opinion [5].

Among all CPGs, only one was specifically developed for the pediatric population; the EAU Guidelines on Pediatric Urology includes a dedicated chapter on the diagnosis and management of children and adolescent varicocele [18]. Notably, children and adolescent varicoceles were included as subsections within the varicocele chapter in the EAU guidelines on male infertility. Similarly, both the AUA and ASRM included the topic of children and adolescent varicocele as a subsection of its guideline on varicocele. The reasons might stem from the relative paucity of information on the matter concerned and reinforces the need for well-designed studies regarding varicocele in this subgroup of patients.

Methods Used to Collect and Select the Evidence

The AUA developed their recommendations based on expert opinion [15]. The EAU guideline was based on a systematic literature search performed by panel members. The search was done covering 2012 and 2013, with a cutoff date of September 2013. Embase, MEDLINE, and the Cochrane Central Register of Controlled Trials were searched, with a limitation to reviews, meta-analysis, or meta-analysis of RCTs [17]. The ESPU guideline was developed after a systematic review using MEDLINE. Application

of a structured analysis of the literature was not possible in many conditions due to a lack of well-designed studies [18]. As for the ASRM guideline, the methods for collecting and selecting the evidence were not stated.

Methods Used to Analyze the Evidence

The EAU guidelines used previously published meta-analyses and systematic reviews [17]. The authors of the ESPU document clearly stated that due to the limited availability of large RCTs, there is a need for continuous re-evaluation of the information provided. This paucity of RCTs makes their document more like a consensus [18]. This information was not stated on AUA and ASRM documents.

Methods Used to Assess the Quality and Strength of the Evidence

These methods were used only on EAU and ESPU documents. Both guidelines graded the references according to their level of evidence (LE), and guidelines are given a grade of recommendation (GR), according to a classification system modified from the Oxford Centre for Evidence-Based Medicine Levels of Evidence [17, 18].

Description of Methods Used to Formulate the Recommendations

The AUA BPS was written by the Male Infertility Best Practice Policy Committee of the AUA, which was created in 1999 by the Board of Directors of the AUA, and the Practice Committee of the ASRM. A working group of 12 members drafted the document [15]. The ASRM recommendations were developed under the direction of the Practice Committee of the ASRM and the Society for Male Reproduction and Urology. A working group of 21 members drafted the document [16]. Most working group members are academic urologists with a particular interest in the topic. Specialists from other medical fields are included as full members of the working group as needed. The recommendations were graded according to the Oxford Centre for Evidence-Based Medicine Levels of Evidence. Availability of RCT may not necessarily translate into a grade A recommendation where there are methodological limitations or disparity in published results. The absence of a high level of evidence did not necessarily preclude a grade A recommendation if there were overwhelming clinical experience and consensus [17]. Finally, a collaborative working group consisting of members representing the European Society for Paediatric Urology (ESPU) and the European Association of Urology (EAU) has prepared these guidelines. This document was peer-reviewed before publication [18].

Method of Guideline Validation

The AUA document was peer-reviewed by 125 physicians and researchers related to infertility. The Practice Committee of the ASRM made modifications, and finally, the document was submitted to and approved by the Board of Directors of the AUA and ASRM [15]. The Practice Committee and the Board of Directors of the ASRM and the Board of the Society for Male Reproduction and Urology have approved the report. ASRM members reviewed the document, and their input was considered in the preparation of the final document [16]. Both EAU and ESPU validated their guidelines based on an external and internal review [17, 18].

Conclusions

The management of varicocele in children and adolescents remains a controversial topic in pediatric urology. The primary reason seems to relate to difficulties in determining the effect of the varicocele in future fertility precisely. Even when using the available guidelines to establish the diagnosis and management of children and adolescents with varicocele, there are significant limitations.

There are notable differences in the methods of these guidelines' development, data collection, and analysis. Moreover, there are differences between the European and American approach to varicocele diagnosis and management. All guidelines stated their objectives and included the intended users and the methods used to develop, to analyze the evidence, as well as to formulate the recommendations. However, the EAU and ESPU guidelines were more detailed regarding diagnosis, and they also provided a broader range of treatment indications when comparing to AUA and ASRM guidelines. Thus, there are ample opportunities to develop more studies and research in this field, aiming in incorporating higher-quality evidence in the management of this critical pathology in a pediatric and adolescent population.

Review Criteria

A thorough search of medical literature was conducted with the MEDLINE, Embase, ScienceDirect, and SciELO databases until November 2018. The electronic database search was supplemented by searching guidelines websites; specifically, we searched "Guidelines International Network" (G-I-N; www.g-i-n.net), "National Guidelines Clearinghouse" (www.guideline.gov), and "National Institute for Health and Clinical Excellence" (NICE; www.nice.org.uk) websites. We used relevant terms, namely, "varicocele," "child," "pediatric," and "adolescent" and "guidelines," "best practice statements," and "committee opinion."

Multiple Choice Questions and Answers

1. Concerning varicocele in adolescents, which of the following is correct?
   (a) Most adolescents with varicocele present with abnormal semen analysis.
   (b) Most adolescents with varicocele have testicular atrophy.
   (c) **Most adolescent varicoceles are asymptomatic.**
   (d) All the above
2. The prevalence of varicocele is:
   (a) Higher than 20% in children and adolescents
   (b) Higher than 10% and lower than 20% in children and adolescents
   (c) **Rarely seen in children and around 15% in adolescents**
   (d) None of the above
3. Concerning diagnosis and treatment of children and adolescent varicocele:
   (a) Treatment is well established and based on level 1 evidence.
   (b) The available guidelines concur on recommendations concerning diagnosis and treatment.
   (c) **Remains controversial**.
   (d) There are no differences compared to adult varicocele.
4. The Dubin and Amelar varicocele grading scale is:
   (a) The most commonly used classification for varicocele and it is based on ultrasound examination
   (b) **The most commonly used classification for varicocele that is based on physical examination, with grades ranging from I to III**
   (c) The most commonly used classification for varicocele that is based on physical examination, with grades ranging from 0 to III
   (d) The most commonly used classification for varicocele that is based on physical examination, with grades ranging from I to IV
5. Concerning varicocele in children and adolescents, the AUA, ASRM, EAU, and ESPU guidelines:
   (a) Suggest that diagnosis is initially performed by physical examination in supine position
   (b) Recommend that diagnosis should be confirmed by ultrasound
   (c) Recommend that ultrasound should be performed only for clarification
   (d) **The ESPU guidelines provide recommendations considering testicular asymmetry**

6. Concerning treatment indications in children and adolescent:
   (a) All guidelines recommend that a semen analysis should be performed before treatment.
   (b) All guidelines recommend treatment only when a bilateral palpable varicocele is present.
   (c) All guidelines recommend treatment only when a symptomatic varicocele is present.
   (d) **All guidelines recommend treatment when there is a palpable varicocele with evidence of testicular hypotrophy.**

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Sperm DNA Fragmentation Testing and Varicocele

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Chak-Lam Cho, Ashok Agarwal, Sandro C. Esteves, and Ahmad Majzoub

Key Points

- High sperm DNA fragmentation is commonly observed in men with varicocele, irrespective of fertility status.
- Varicocele treatment is effective in alleviating sperm DNA fragmentation in the majority of patients with high sperm DNA fragmentation.
- Greater improvement in sperm DNA fragmentation after varicocele repair predicts better pregnancy outcomes.
- Sperm DNA fragmentation testing has a potential value in better selection of patient who will benefit from varicocele treatment.
- Sperm DNA fragmentation testing should be recommended in patients with grade 2 or 3 varicocele with normal conventional semen parameters and patients with grade 1 varicocele with borderline/abnormal conventional semen parameters.

Introduction

Male infertility attributes to approximately 50–60% of all infertility cases, and varicocele alone accounts for 35% [1]. Varicoceles are considered the most common surgically correctable cause of male factor subfertility. Current evidence supports the role of varicocele treatment which results in improvement in semen parameters and natural pregnancy in 60–80% and 20–60% of couples, respectively [2]. Despite the clear association between varicocele and male subfertility, the poor predictive value of conventional semen parameters on male fertility potential hinders the selection of appropriate patients who will benefit from the treatment of varicocele [3].

The pivotal role of sperm DNA fragmentation (SDF) has been increasingly recognized in the pathophysiology of varicocele-associated infertility [4]. SDF testing offers an opportunity

C.-L. Cho (✉)
S.H. Ho Urology Centre, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong

A. Agarwal
American Center for Reproductive Medicine, Department of Urology, Cleveland Clinic, Cleveland, OH, USA

S. C. Esteves
ANDROFERT, Andrology & Human Reproduction Clinic, Campinas, SP, Brazil

A. Majzoub
Department of Urology, Hamad Medical Corporation, Weill Cornell Medicine Qatar, Doha, Qatar

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to investigate the important genetic content of male gamete that is passed on to the subsequent generations [5]. The potential value of SDF as an independent attribute of semen quality in addition to conventional semen analysis in the evaluation of infertile male has been acknowledged in the latest American Urological Association and European Association of Urology guidelines [6, 7]. Although a precise understanding of the specific utility of SDF testing in different clinical scenarios is still lacking, a guideline has been published recently by the Society for Translational Medicine addressing several specific indications for SDF testing clinically based on the current best evidence [8].

In this chapter, current evidence on the association between varicocele and SDF will be discussed. The relationship will be further illustrated by the effect of varicocele treatment on alleviation of high SDF. Lastly, clinical practice guidelines for SDF testing in varicocele is proposed.

Association Between Varicocele and Sperm DNA Fragmentation

Varicocele remains one of the most controversial topics in medicine. Although a number of etiologies have been proposed, none of them can fully explain the pathophysiology of varicocele-associated infertility. The picture became clearer over the last decades with an understanding of reactive oxygen species (ROS) and oxidative stress (OS), and their negative impact on spermatozoa. The close relationship between varicocele and OS was demonstrated by the higher level of ROS in infertile men with varicocele than infertile men without varicocele [9]. Several studies have also shown that fertile men with varicocele are more likely to have elevated OS in the reproductive tract compared to their counterpart without varicocele [10]. Subsequently, elevated ROS and OS are considered the major cause of SDF. The extent of SDF is a balance between OS and the inherent sperm susceptibility to DNA damage [11]. The hypothesis was supported by the relationship among SDF, ROS production, and efficiency of sperm chromatin protamination in semen samples [12, 13].

The clinical implication of SDF on male fertility became evident as illustrated by the association between high SDF and failure to achieve natural pregnancy [14]. In first pregnancy planners with no previous knowledge of their fertility capability, a high proportion of sperm exhibiting DNA damage correlated with a longer time to achieve natural pregnancy in addition to lower fertility potential compared with low SDF [15]. The significance of SDF testing as a complementary test to semen analysis is best demonstrated by the higher SDF in infertile men with otherwise normal semen parameters compared to fertile controls [16].

Efforts were made to investigate the association between varicocele and SDF in view of the emerging role in SDF testing and understanding in pathophysiological role of SDF in male infertility. Interest in SDF testing for men with varicocele sparkled after a significantly positive association with varicocele was detected in early reports. Sixteen infertile men with left varicocele had been shown to have significantly higher DNA fragmentation index (DFI) than fertile controls (25% vs 15%) [17]. Similar result was reported in another study involving 55 infertile men with clinical varicocele and 25 normozoospermic donors with unknown fertility. The study indicated a significant increase of DNA-damaged spermatozoa in patients with varicocele either with normal or with abnormal conventional semen parameters [18]. The prevalence of SDF in varicocele patients was further summarized by two systematic reviews. In a literature review including 16 case-control studies, overall higher SDF were noted in infertile men with varicocele than those without varicocele in four out of nine studies. In six of seven studies which involved fertile men, SDF were higher in men with varicocele than in fertile men or sperm donor without varicocele, suggesting that varicocele is associated with DNA damage even when fertility has not been compromised [19]. Another systematic review and meta-analysis recruited data from 7 studies including 240 patients with varicocele and

176 controls without varicocele. A significantly higher SDF was observed in patients with varicoceles than controls, with a mean difference of 9.84% (95% CI, 9.19 to 10.49; $P < 0.00001$) [20].

More recent studies provide further data supporting the association between varicocele and elevated SDF and, more importantly, shed light on possible pathophysiological mechanism underlying varicocele-associated male subfertility. The damage to epididymis, in addition to testis, had been suggested in patients with varicocele. It was illustrated by a reduction in neutral α-glucosidase enzyme activity by the epididymis, which was correlated with quality of sperm membrane and nucleus, in men with varicocele. The reduction of enzyme level was correlated with elevated SDF and impaired semen parameters [21]. In another study involving 593 men who attended infertility clinic, SDF was among the highest in men with varicocele (35.7 ± 18.3%) compared to infertile men with other etiologies and fertile controls. In addition, two distinct sperm populations with fragmented DNA were identified, namely standard and degraded DNA fragmentation. The study suggested that the proportion of sperm with degraded DNA which signified massive DNA breaks was eightfold higher in varicocele patients than sperm donors. The utilization of degraded DNA fragmentation index may possibly identify varicocele patients with 94% accuracy [22]. Moreover, higher SDF and significantly lower expression of PLCζ was observed in infertile men with varicocele compared to fertile controls. The result suggested that PLCζ, which is one of the main sperm factors involved in oocyte activation, may be one of the mediators between varicocele and reduced male fertility [23].

Association between varicocele and elevated SDF can also be demonstrated in adolescents which may affect management decision. The result of SDF testing may objectively illustrate testicular dysfunction which may predict possible progression to infertility and select patients who will benefit from early varicocele treatment. Adolescents with bilateral grade 2 or 3 varicoceles were compared to counterparts without a varicocele in a study. The study suggested that adolescents with varicocele had an increase in SDF despite the lack of difference in standard semen analysis between the groups [24].

Collectively, association between varicocele and high SDF was supported by both studies on pathophysiology and clinical data. A significantly higher SDF was observed in adults and adolescents with varicocele, irrespective of fertility status and conventional semen parameters. The strong correlation between SDF and male fertility signifies the potential value of SDF testing in assessment of patients with varicocele and selection of appropriate candidates for varicocele treatment.

Implication of Varicocele Treatment on Sperm DNA Fragmentation

Intervention for varicocele has been widely accepted in the management of male infertility [25]. Compelling evidence is available to suggest the role of varicocelectomy in improving semen parameters and pregnancy rates in infertile male [26, 27]. Recently, alternate indications for varicocele repair has been recognized with increasing knowledge of implication of SDF and OS on male fertility. The effect of varicocele treatment in alleviation of SDF was reported by a number of studies over the past two decades as summarized in Table 57.1. This further validates the role of SDF testing in management decision of patients with varicocele.

Majority of early retrospective cohorts reported significant reduction in SDF 3–6 months after microsurgical subinguinal varicocelectomy (MSV) in 78–90% of patients with clinical varicocele [9, 28–31]. Treatment effect of varicocelectomy on SDF in adolescents with grade 2 or 3 varicoceles has also been reported in a prospective cohort including 21 patients aged between 15 and 19 [32]. The results of early studies (9 prospective and 3 retrospective) involving 511 patients were later reviewed. A reduction in sperm damage after varicocelectomy was reported by all studies comparing patients with varicocele to control group, irrespective of different assay methodologies [19]. Another meta-analysis of seven stud-

Table 57.1 Summary of studies evaluating the effect of varicocelectomy on sperm DNA fragmentation

Study	Design	Patients	Controls	SDF assay	Results
Zini (2005) [28]	Retrospective cohort	37 patients with varicocele who had microsurgical subinguinal varicocelectomy performed	N/A	SCSA	Mean SDF decreased after varicocelectomy (pre, 27.7%; post, 24.6%; $P = 0.04$)
Sakamoto (2008) [9]	Retrospective cohort	30 infertile men with grade 2 or 3 varicocele (15 oligozoospermic and 15 normozoospermic) who had microsurgical subinguinal varicocelectomy performed	N/A (TUNEL result of controls was not provided)	TUNEL	TUNEL-positive sperm decreased significantly 6 months after treatment (pre, 79.6%; post, 27.5%; $P < 0.001$)
Werthman (2008) [29]	Retrospective cohort	11 patients with clinical varicocele and DFI >27% who had microsurgical subinguinal varicocelectomy performed	N/A	SCSA	10 of the 11 patients showed a significant decrease in SDF 3–6 months after varicocelectomy 7 of 11 patients showed decrease in DFI to normal level, and the mean percent change in DFI was 24%
Moskovtsev (2009) [30]	Retrospective cohort	Patients with clinical varicocele was treated with oral antioxidants alone (37 men), or subjected to both microsurgical subinguinal varicocelectomy and oral antioxidants (9 men)	N/A	SCSA	SDF decreased in 78% of patients subjected to both varicocelectomy and oral antioxidants (pre, 44.7%; post, 28.4%; $P < 0.03$) No improvement in SDF was observed in patients on oral antioxidants alone (pre, 45.3%; post, 42.5%)
Smit (2010) [31]	Prospective cohort	49 patients with clinical varicocele and oligozoospermia who had high inguinal ligation (36 men) or microsurgical varicocelectomy (8 men) performed	N/A	SCSA	Improvement in SDF was observed after treatment (pre, 35.2%; post, 30.2%; $P = 0.019$) 37% of couples conceived naturally, and 24% achieved pregnancy with assisted reproduction after treatment Mean postoperative DFI was significantly lower in couples who conceive naturally or with assisted reproduction than those who did not (spontaneous pregnancy, 30.1% vs 37.5%; assisted reproduction, 21.3% vs 36.9%)
Zini (2011) [19]	Prospective cohort	25 patients with clinical varicocele and abnormal semen parameters who had microsurgical subinguinal varicocelectomy performed	N/A	SCSA	Improvement in SDF was observed at 4 and 6 months after varicocelectomy (pre, 18%; 4 months, 10%; 6 months, 7%)

Lacerda (2011) [32]	Prospective cohort	21 adolescents (age 15–19) with grades 2 or 3 varicocele who had microsurgical subinguinal varicocelectomy performed	N/A	Comet	Sperm with intact nuclear DNA (Comet class I) increased after varicocelectomy (49.6 to 64.5%; $P = 0.011$)
La Vignera (2012) [34]	Not specified	30 patients with grade 3 left varicocele and oligoasthenoteratozoospermia who had microsurgical subinguinal varicocelectomy performed	30 normozoospermic controls without varicocele	TUNEL	Significant reduction in SDF at 4 months after varicocelectomy (5.0 to 2.1%; $P < 0.05$), and postoperative results were similar to that of healthy controls (2.0%)
Li (2012) [35]	Not specified	19 patients with clinical varicocele who had microsurgical subinguinal varicocelectomy performed	19 normozoospermic men	SCSA	SDF was higher in men with varicocele than controls (28.4% vs 17.4%; $P = 0.007$) DFI decreased 3 months after operation (28.4 to 22.4%; $P = 0.018$), and postoperative results were similar to that of controls
Baker (2013) [36]	Retrospective cohort	24 patients with clinical varicocele who had microsurgical subinguinal varicocelectomy performed	N/A	TUNEL	SDF decreased after varicocelectomy (40.8 to 24.5%) A higher preoperative SDF was associated with a larger improvement postoperatively Postoperative SDF in pregnant and nonpregnant couples showed no difference (22.2% vs 25.7%)
Kadioglu (2014) [37]	Retrospective cohort	92 infertile patients with clinical left varicocele and abnormal semen analysis who had microsurgical subinguinal varicocelectomy performed	N/A	TUNEL	SDF decreased 6 months after varicocelectomy (42.6 to 20.5%; $P < 0.001$) A higher preoperative SDF was associated with a larger improvement postoperatively
Ni (2014) [38]	Prospective cohort	42 infertile men with clinical left varicocele and abnormal semen parameters who had microsurgical varicocelectomy performed	10 normozoospermic fertile controls	SCSA	Higher DFI was observed in preoperative group compared to controls (27.4% vs 11.5%; $P < 0.01$) DFI in patients who achieved pregnancy (20.6%) were lower than preoperative value (27.4%) and those of nonpregnant patients (24.7%) DFI in patients who achieved pregnancy after varicocelectomy were not significantly different from controls (20.6% vs 11.5%)

(continued)

Table 57.1 (continued)

Study	Design	Patients	Controls	SDF assay	Results
Pourmand (2014) [39]	Randomized controlled trial	100 infertile patients with clinical left varicocele or subclinical varicocele who had varicocelectomy alone (group 1) or varicocelectomy plus oral L-carnitine for 6 months (group 2)	N/A	TUNEL	Improvement in SDF was observed in both groups after varicocelectomy (group 1, 14.0 to 9.5%; group 2, 13.9 to 8.5%) The results were not different between groups
Telli (2015) [40]	Prospective cohort	72 infertile patients with clinical varicocele and oligozoospermia who had macroscopic inguinal varicocelectomy performed	N/A	Acridine orange assay	SDF decreased after varicocelectomy (34.5 to 28.2%) with a mean follow-up of 6.2 months
Tavalaee (2015) [41]	Not specified	23 infertile patients with grades 2 or 3 left varicocele who had varicocelectomy performed	N/A	TUNEL	SDF improved 3 months after varicocelectomy (15.9 to 10.8%; $P < 0.001$)
Mohammed (2015) [42]	Prospective cohort	75 infertile patients with clinical varicocele and altered semen parameters who had subinguinal varicocelectomy performed with loop magnification	40 healthy fertile volunteers without varicocele	Acridine orange	Higher DFI was observed in preoperative patients than controls (32.4% vs 18.2%; $P = 0.003$) DFI deceased significantly after varicocelectomy (32.4 to 20.0%; $P = 0.05$) DFI in patients who achieved pregnancy at 1 year were significantly lower than those who did not (16.4% vs 24.2%; $P = 0.04$)
Alhathal (2016) [43]	Prospective cohort	29 infertile patients with clinical varicocele and abnormal semen parameters who had microsurgical subinguinal varicocelectomy performed	6 healthy sperm donor with normal semen parameters	SCSA	DFI was significantly higher in preoperative patients than controls (20.0% vs 7.4%; $P = 0.01$) DFI improved significantly after varicocelectomy (20.0 to 12.0%; $P = 0.001$)
Ni (2016) [44]	Not specified	51 patients with clinical varicocele and abnormal semen analysis who had microsurgical retroperitoneal high ligation performed	15 men with subclinical varicocele, 22 men with clinical varicocele and normozoospermia, and 25 healthy fertile donors	SCSA	SDF was higher in patients with clinical varicocele (range, 20.6–30.0%) compared to patients with subclinical varicocele (14.9%) and controls (12.0%) SDF reduced in patients with clinical varicocele and altered semen parameters, irrespective of clinical grade of varicocele SDF was lower in patients who achieved pregnancy than nonpregnant patients

Abdelbaki (2017) [45]	Prospective controlled cohort	60 infertile patients with clinical varicocele and abnormal semen parameters who had inguinal varicocelectomy performed with loop magnification	20 normozoospermic healthy fertile men	SCSA	A higher DFI was observed in patients with varicocele than controls (29.9% vs 7.6%) DFI improved 3 months after varicocelectomy (29.9 to 18.8%; $P < 0.001$)
Zaazaa (2018) [46]	Randomized controlled trial	80 infertile patients with clinical grade 2 or 3 varicocele and DFI >30% who had varicocelectomy (group 1), or varicocelectomy followed by ketotifen 1 mg twice daily for 3 months (group 2) 40 men with clinical grade 2 or 3 varicocele and DFI >30% treated with oral ketotifen 1 mg twice daily for 3 months (group 3)	N/A	SCD	DFI improved after 3 months of treatment in all groups DFI improvement was the highest in patients with varicocelectomy followed by mast cell stabilizer (26.8%) compared with varicocelectomy alone (18.2%; $P = 0.04$) and mast cell stabilizer alone (16.8%; $P = 0.02$) Improvement in DFI was higher in infertile patients with grade 3 varicocele compared to those with grade 2
Sun (2018) [47]	Randomized controlled trial	358 infertile patients with left clinical and right subclinical varicocele who had either unilateral left or bilateral microsurgical subinguinal varicocelectomy performed	N/A	SCSA	DFI was significantly reduced in both groups 1 year after varicocelectomy (unilateral, 21.6 to 11.8%; bilateral, 23.0 to 12.1%) No difference in preoperative and postoperative DFI between the two groups was observed, despite greater improvement in semen parameters in the bilateral group

DFI DNA fragmentation index, *N/A* not applicable, *SCD* sperm chromatin dispersion, *SCSA* sperm chromatin structure assay, *SDF* sperm DNA fragmentation, *TUNEL* terminal deoxynucleotidyl transferase dUTP nick end labeling

ies further illustrated an improvement in sperm DNA integrity after varicocele with a mean difference in SDF of 3.37% (95% CI, 2.65, 4.09; $P < 0.00001$) compared to no treatment [20].

More recent studies have reported similar results in reduction of SDF after varicocele treatment and further assessed the impact of this reduction on pregnancy rates [33]. Smit et al. examined 49 patients who had a 1-year history of infertility and who underwent varicocelectomy. SDF assessed by sperm chromatin structure assay (SCSA) 3 months after the operation significantly decreased from 35.2% to 30.2%. Natural pregnancy was reported in 37% of patients and a further 24% achieved pregnancy with assisted reproduction. The authors observed that SDF was significantly lower in couples who conceived naturally or with assisted reproduction compared to those who did not [31]. In another study, Ni et al. evaluated 43 infertile men with grade 2/3 varicocele and altered semen parameters and 10 normozoospermic fertile controls. Although SDF remained higher in patient group than in control group pre- and postoperatively, there was a significant overall reduction of SDF 3–6 months after microsurgical varicocelectomy (preoperative (28.4%) vs postoperative (22.4%); $P = 0.018$). Notably, similar conclusion has been reached when comparing postoperative SDF results of pregnant and nonpregnant patients. SDF in patients who achieved pregnancy after varicocelectomy (20.6% ± 3.5%) was not significantly different from controls (11.5% ± 3.9%) but was lower than both preoperative values (27.4% ± 6.3%; $P < 0.01$) and the results of nonpregnant patients (24.7% ± 6.5%; $P < 0.01$) [44]. Mohammed et al. compared 75 infertile patients with clinical varicocele and impaired semen parameters who had subinguinal varicocelectomy performed to 40 healthy fertile controls without varicocele. They reported higher DFI in preoperative patients than controls (32.4% vs 18.2%; $P = 0.003$) and a significant decrease in DFI after varicocelectomy in patient group (32.4% to 20.0%; $P = 0.05$). A lower DFI was found in patients who achieved pregnancy at 1 year than those who did not (16.4% vs 24.2%; $P = 0.04$) [42]. Recently, Abdelbacki et al. prospectively evaluated 60 infertile patients with clinical varicocele and abnormal semen parameters. DFI was identified as one of the preoperative variables that predicted success after inguinal varicocelectomy by a logistical regression model comparing infertile patients to healthy fertile controls. Every 1% increase in DFI correlated with a decreased chance of improvement in fertility post-varicocelectomy by a factor of 1.4. This important study illustrates the potential role of preoperative SDF in concordance with fertility potential and treatment success [45]. On the other hand, not all studies support the positive impact of SDF testing in prediction of pregnancy outcomes. Baker et al. retrospectively reviewed pre- and postoperative SDF results of 24 infertile patients with clinical varicocele and MSV performed. Despite a significant decrease in SDF after repair (40.8 to 24.5%) and larger improvement in patients with higher preoperative SDF, SDF results in pregnant and nonpregnant couples did not differ in contrast to previous studies (22.2% vs 25.7%) [36].

The association between subclinical varicocele and SDF, and the implication of repair of subclinical varicocele on SDF is less studied with limited evidence available. SDF rates of 60 infertile patients with varicocele were studied using various assay methods. While patients with subclinical varicocele demonstrated substantial SDF as in patients with clinical varicocele, only patients with clinical varicocele experienced improvement in SDF rates after surgery [48]. Another study compared 358 infertile men with left clinical and right subclinical varicoceles who had either unilateral left or bilateral MSV. Despite a greater improvement in semen parameters was demonstrated in the bilateral group, there was no difference in pre- and postoperative DFI between the two groups [47]. As a result, repair of subclinical varicocele, both in the settings of unilateral subclinical varicocele and concomitant with contralateral clinical varicocele, in alleviation of SDF is not supported by current evidence.

The role of other treatment modalities on SDF remains largely unknown and the published literature is very scarce. A study retrospectively

analyzed data of 46 men with clinical varicocele. While significant decrease in SDF was reported in the majority of patients subjected to varicocelectomy and oral antioxidants, no improvement in SDF was observed in patients on oral antioxidants alone [30]. Similar results was reported in a randomized controlled trial comparing 100 infertile patients with varicocele who had varicoceletomy alone or varicocelectomy plus oral L-carnitine for 6 months. No difference in improvement of SDF could be identified between the groups [39]. After the initial negative studies, further studies shed light on the potential positive impact of oral therapy in the management of high SDF, either alone or as an adjunct to surgery. In a noncontrolled study, 20 patients with grade 1 varicocele were treated with a combination of oral antioxidants for 3 months. The relative reductions in SDF and the percentage of highly degraded sperm were 22.2% (P = 0.02) and 31.3% (P = 0.07), respectively [49]. More recently, improvement in DFI was superior in patients after varicocelectomy followed by mast cell stablizer compared to those who received either treatment alone. The rationale of using mast cell stablizer is based on the finding of increased testicular mast cells in close proximity to seminiferous tubules which promotes the release of inflammatory mediators and dysfunction of the blood-testis barrier. The result of this randomized controlled trial involving 120 men with clinical grade 2 or 3 varicocele and DFI >30% supported the use of oral medications as adjunctive treatment to surgical intervention [46]. The current evidence is too limited and often contradictory. Large well-designed studies on potential role of oral therapy as a possible therapeutic strategy for high SDF in men with varicocele are required before any definitive conclusion can be drawn.

In summary, emerging evidence certainly support the role of varicocele repair in alleviation of high SDF in the majority of infertile men with clinical varicocele. The relationship between post-varicocelectomy SDF and pregnancy outcomes reported repeatedly from studies provides strong evidence to support the potential use of SDF testing in evaluation of men with varicocele. Repair of subclinical varicocele and use of oral treatments is not recommended based on current evidence. Varicocelectomy represent a viable treatment option for infertile male with clinical varicocele and high SDF. Counseling of infertile couples about the relationship between SDF and infertility before treatment decision is of paramount importance.

Clinical Practice Guidelines for Sperm DNA Fragmentation Testing in Varicocele

The role of SDF testing in clinical practice remains poorly defined irrespective of the increasing understanding of the implication of SDF on male infertility. Although there is insufficient evidence to support the routine use of SDF testing in evaluation of infertile men [6], a clinical practice guideline on SDF testing is of great value in translating the best evidence into clinical practice and serve as a framework for standardized care and future research. Indeed, a recently published clinical practice guideline issued by the Society for Translational Medicine represents the first attempt to suggest specific indications for SDF testing in male infertility scenarios including varicocele [8].

Current guidelines issued by major professional societies recommend varicocele repair in infertile men with clinical varicocele and abnormal semen parameters [6, 7]. The clinical practice guideline for SDF testing expanded the indication and recommended varicocelectomy to be considered in patients with high SDF and (i) grade 2 or 3 varicocele and normal conventional semen parameters; (ii) grade 1 varicocele with borderline/abnormal conventional semen parameters based on WHO criteria (Table 57.2) [8]. The recommendations is based on the association between SDF and varicocele, and the reversible nature of high SDF in the majority of patients after varicocelectomy as discussed in previous sections. The utility of SDF testing in patients with clinical varicocele facilitates better selection of varicocelectomy candidates, counselling of infertile couples on reproductive outcomes, and monitoring of treatment progress.

Table 57.2 Clinical practice guidelines issued by the Society for Translational Medicine on indications of sperm DNA fragmentation testing in patients with clinical varicocele

SDF testing is recommended in patients with grade 2 or 3 varicocele with normal conventional semen parameters
SDF testing is recommended in patients with grade 1 varicocele with borderline/abnormal conventional semen parameter results

Based on data from Ref. [8]
SDF sperm DNA fragmentation

Conclusion

The use of semen analysis in patients with varicocele does not necessarily predict treatment outcome. There is a need for new diagnostic tests with greater precision in identifying appropriate candidates for treatment. Sperm DNA fragmentation testing has gained popularity in the last decade as a possible tool to assess fertility. Emerging evidence demonstrates the association between clinical varicocele and high SDF and the reduction in SDF after varicocele repair. The findings support the utilization of SDF testing in patients with varicocele. Although the evidence is still limited and warrants further research, the current best evidence has been summarized in clinical practice guidelines as it offers recommendations for use of SDF testing in patients with clinical varicocele.

Review Criteria

An extensive search of studies investigating the relationship between varicocele and sperm DNA fragmentation was performed using search engines including ScienceDirect, OVID, PubMed, and MEDLINE. The study identification was based on the following keywords: "varicocele," "varicocelectomy," "varicocele treatment," "sperm DNA fragmentation," and "sperm DNA damage." The start and end dates for the searches were January 2000 to September 2018, respectively. Only articles published in English were considered. Data that were solely published in conference or meeting proceedings, websites or books were not included.

Multiple Choice Questions and Answers

1. The highest sperm DNA fragmentation is observed in:
   (a) Fertile men with varicocele
   (b) **Infertile men with varicocele**
   (c) Fertile men without varicocele
   (d) Infertile men without varicocele
2. Which of the following statement is correct?
   (a) Sperm DNA fragmentation is absent in sperm donors.
   (b) Sperm DNA fragmentation is higher in men with normal conventional semen parameters.
   (c) **Sperm DNA fragmentation is higher in men with varicocele compared to those who do not have the condition.**
   (d) Men with varicocele and high sperm DNA fragmentation are infertile.
3. The potential value(s) of sperm DNA fragmentation testing in patients with clinical varicocele includes:
   (a) Better selection of surgical candidates for varicocele treatment
   (b) Monitoring of progress after varicocele treatment
   (c) Counselling of patients on reproductive outcomes
   (d) **All of the above**

4. Sperm DNA fragmentation testing should be recommended in patients with:
   (a) High-grade varicocele and abnormal conventional semen parameters
   (b) **High-grade varicocele and normal conventional semen parameters**
   (c) Low-grade varicocele and normal conventional semen parameters
   (d) Bilateral subclinical varicoceles

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Grade of Evidence on Varicocele Treatment

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Guilherme J. A. Wood
and Marcello Antonio Signorelli Cocuzza

Key Points

- Varicocelectomy may benefit infertile couples seeking natural conception by improving sperm parameters, reducing sperm DNA fragmentation, and reducing miscarriage rates.
- Varicocele surgical treatment is an effective treatment for painful varicocele, especially on patients reporting longer-duration pain.
- Varicocele is detrimental to testosterone levels, and hypogonadal subfertile patients may be benefited by varicocele treatment.
- Patients submitted to assisted reproduction technologies may be benefited by varicocelectomy, by potentially lowering treatment complexity, higher live birth rates, and increased sperm retrieval rates in azoospermic men.
- To this date, there is no evidence that surgical treatment of subclinical varicocele improves pregnancy rates on infertile couples.

Introduction

The deleterious effects of varicocele on male infertility were first described in 1955, when Tulloch first reported improvement of sperm parameters and successful paternity after varicocele ligation [1]. After more than six decades of medical research, however, varicocele treatment remains one of the most controversial subjects in the field of urology. Contributing to this lack of controlled studies are precise diagnostic parameters and treatment guidelines along with progressive decrease in the interest on male infertility research and treatment after the advent of intracytoplasmic sperm injection (ICSI).

Nevertheless, numerous researches investigated the effects of varicocele treatment on human reproduction. In this chapter, we aim to summarize the existing literature regarding the efficacy of varicocelectomy through the scope of evidence-based medicine.

Varicocele Repair in Infertile Adults

Varicocele is the most common identifiable cause of male infertility [2]. The prevalence of varicocele is higher in infertile patients; while present in 4.4 to 22.6% of adult males, it can be diagnosed in 21 to 41% of men with primary infertility and in 75 to 81% of men suffering from secondary infertility [2–4]. Several studies have

G. J. A. Wood · M. A. S. Cocuzza (✉)
Department of Urology, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil
e-mail: mcocuzza@uol.com.br

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tried to address the benefits of varicocelectomy on seminal parameters and pregnancy rates; however, due to the ethical aspects of conducting a randomized controlled trial (RCT) on surgical patients, few high-quality data exist on the subject.

Two recent RCTs were published regarding this issue. In 2010, Abdel-Meguid et al. randomized 150 infertile patients with palpable varicoceles to observation or subinguinal microsurgical varicocelectomy [5]. After 12-month follow-up, natural pregnancy was higher in the intervention group (32.9% vs. 13.9%), resulting in an odds ratio (OR) of 3.04. All semen parameters also improved after surgery while remaining stable in the observation group.

Ghanaie et al. published in 2011 the results from a RCT in which 136 patients with normal semen parameters were randomized to varicocelectomy or expectant therapy [6]. In this study, all sperm parameters improved significantly after surgery. Additionally, pregnancy rate was higher in the group of patients submitted to surgery (44.1% vs. 19.1%, $p = 0.003$), and miscarriage rates were higher in patients in the expectant treatment group (69.2% vs. 13.3%, $p = 0.001$).

In 1998, a randomized interventional trial conducted by Barbalias et al. reported improvement of sperm concentration and motility following varicocelectomy, regardless of the surgical technique used, but the authors failed to obtain a control group due to patients' refusal [7]. Pregnancy rates were not reported in this study.

Varicocelectomy may be beneficial to semen parameters of patients without fertility issues as well. Cho et al. published in 2010 a study reporting results from patients treated because of non-infertility-related symptoms (testicular pain, discomfort, or enlarged scrotal volume), with at least one abnormal semen parameter [8]. All 121 patients were submitted to microsurgical subinguinal varicocelectomy, and an improvement of at least one semen parameter was observed in 76% of the patients. Sperm morphology, however, did not improve after surgery.

In a meta-analysis published in 2007, Agarwal et al. concluded that surgical varicocelectomy results in improvement of sperm parameters of infertile men with clinical varicocele [9]. The 17 studies included in this analysis did not have a control expectant group—individuals served as their own controls. Sperm concentration improved in average by 9.71 to 12.03 million per mL, while sperm motility improved by approximately 10%. In this meta-analysis, there was also a statistically significant improvement of 3.16% in sperm morphology after surgery.

In 2011, another meta-analysis published by Schauer et al. including 14 articles concluded that varicocelectomy results in significant or highly significant improvement of sperm count and motility [10]. The average increase in sperm count was approximately 9 million per mL, regardless of the surgical technique used. Sperm morphology, however, did not improve after surgery.

DNA fragmentation index (DFI) is also proposed to improve after varicocele repair. In 2009, Smit et al. published results from a prospective study that included 49 patients submitted to varicocelectomy [11]. After the procedure, DFI mean decreased from 35.2% to 30.2% ($p = 0.019$). In a subgroup analysis, patients were divided into responders and non-responders to varicocele repair, which was defined as improvement of at least 50% of sperm concentration ($n = 31$ and 18 patients, respectively). DFI mean decrease was greater in the responders group, from 35.3% to 28.6% ($p = 0.009$).

Two Cochrane systematic reviews addressed the benefits of varicocelectomy on pregnancy rates. In 2004, after reviewing eight RCTs, Evers and Collins concluded that varicocele treatment provided no benefit over expectant management in subfertile couples [12]. This review, however, included heterogeneous studies, and three of the eight studies specifically included only patients with subclinical varicocele. In 2012, Kroese et al. published an updated meta-analysis, this time excluding from the analysis studies that involved men with normal semen analysis, azoospermia, and subclinical varicocele [13]. This subgroup analysis comprised five trials and favored treatment versus non-treatment, with an OR of 2.39 for the pregnancy rate and a number needed to treat of 7. The overall quality of evidence was rated as low.

Table 58.1 Varicocelectomy in infertile adults

Author	Year	Level of evidence	
Infertile Male			
Ghanaie et al.	2012	1b	Individual RCT
Kroese et al.	2012	1a	SR of RCT
Miyaoka et al.	2012	–	Narrative review
Schauer et al	2012	1a	SR of RCT
Abdel-Meguid et al.	2011	1b	Individual RCT
Cho et al.	2011	3b	Individual case-control study
Smit et al.	2010	2b	Individual cohort study
Agarwal et al.	2007	1a	SR of RCT
Evers et al.	2004	1a	SR of RCT
Barbalias et al.	1998	2b	Individual low-quality RCT
Gorelick et al.	1993	2b	Exploratory cohort study
Kursh et al.	1987	2b	Exploratory cohort study

SR systematic review.
RCT randomized controlled trials.
Levels of evidence based on data from https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/

Scientific evidence on this subject comes from RCTs and high-quality meta-analysis. Therefore, varicocele repair on infertile patients is recommended, with evidence level 1a (Table 58.1). In conclusion, varicocelectomy is very likely to benefit couples seeking fertility. Most studies have shown improvement of sperm parameters following varicocelectomy in patients with clinical varicocele, and pregnancy rates are higher after varicocele treatment in patients with clinical varicocele and low sperm parameters. Patients with normal sperm parameters, however, might not benefit from treatment regarding pregnancy rates.

Varicocele and Testicular Pain

The incidence of testicular pain caused by varicocele is uncommon—previous studies estimate that it will be present in only 2–10% of patients with varicocele [14]. Testicular pain caused by varicocele is usually described as a dull throbbing pain in the testicle or spermatic cord, or scrotal heaviness that worsens with exercise or prolonged upright position. To date, there are no randomized studies investigating the outcomes of varicocelectomy on testicular pain.

Data from one prospective study and several retrospective studies report complete resolution of pain rates ranging from 53 to 90%, and partial improvement of pain rates from 84 to 100% [15]. In 2012, Abd Ellatif et al. presented results from a prospective study in which 152 patients presenting with testicular pain and varicocele were included [16]. Seven patients (4.6%) reported symptoms improvement with conservative treatment, and 145 patients were submitted to varicocelectomy. Of the 130 patients available after 3 months follow-up, 109 (83.8%) reported complete remission of pain and 7 (5.4%) reported partial resolution of pain. No differences were observed regarding varicocele ligation type or varicocele grade. Pain duration shorter than 6 months was related to worse outcomes in pain improvement (93.3% vs. 78%).

A retrospective study published by Altunoluk et al. in 2010 reported similar results [17]. Two hundred eighty-four patients were submitted to microsurgical subinguinal varicocelectomy and were divided into two groups according to the duration of symptoms (greater or shorter than 3 months). Success rates were higher for patients reporting long-lasting pain (98.6% vs. 82.3%). Treatment failure was not associated to varicocele grade.

A study published by Chen et al. in 2012 including 76 patients with unilateral varicocele addressed factors that could predict pain resolution success [18]. Body mass index, sex steroids, and maximal vein diameter were not associated with negative outcomes. On the other side, greater number of veins, higher preoperative pain score and longer duration of pain was associated with better pain resolution in 6-month follow-up.

Overall, varicocelectomy is an effective treatment for painful varicocele. While patients reporting longer-duration pain showed better outcomes. Levels of evidence of this topic are summarized in Table 58.2, with most of the studies with level of evidence 2 and 3. Further investigation is needed to define if higher varicocele grade influence success rates.

Table 58.2 Varicocelectomy and testicular pain

Author	Year	Level of evidence	
Testicular Pain			
Paick et al.	2018	–	Narrative review
Abd Ellatif et al.	2012	2b	Individual cohort study
Chen et al.	2012	3a	SR of case-control studies
Altunoluk et al.	2010	3b	Individual case-control study
Peterson et al.	1998	2c	Outcome research

SR systematic review
RCT randomized controlled trials
Levels of evidence based on data from https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/

Varicocele Repair in Hypogonadic Males

The relationship between varicocele and Leydig cell dysfunction has been studied for decades. Nevertheless, the known association between male infertility and lower testosterone levels make this assumption more difficult to prove. Several studies, however, indicate that varicocele may cause some impairment of testicular endocrine functions, resulting in increased Leydig cells apoptosis, lower intratesticular testosterone levels, and altered histological patterns [19, 20].

Those findings are likely to be responsible for lower testosterone levels on varicocele patients. Tanrikut et al. found in 2011 that men with varicocele had lower preoperative testosterone levels when compared to controls without varicocele (416 vs. 469 ng/dL) [21]. Varicocele patients, however, were younger than controls in this study, but the difference persisted after age-controlled statistical analysis. Another study published in 2007 by Hurtado de Catalfo et al. achieved similar results. Testosterone levels were approximately 25% lower in infertile males with left varicocele than in fertile controls [22].

In a prospective controlled non-randomized study conducted by Abdel-Meguid et al. in 2014, varicocelectomy resulted in 12.9% mean improvement of testosterone levels in infertile men with varicocele (44.7 ng/dL, $p < 0.0001$) [23]. Controls, composed of groups of varicocele-infertile men, varicocele-fertile men, and normal controls, did not exhibit significant changes in baseline testosterone. In a subgroup analysis, testosterone improvement was significant in previously hypogonadal patients (93.7 ng/dL; 40.1%, $p < 0.0001$) and nonsignificant in eugonadal patients, however. A meta-analysis comprising 7 studies published in 2017 by Chen et al. showed a mean increase of postoperative testosterone levels of 34.3 ng/dL [24]. Once again, testosterone improvement was higher in hypogonadal patients when compared to eugonadals and untreated patients.

Thus, it is possible to infer that varicocele is prejudicial to Leydig cell function, and surgical correction may diminish this effect. Table 58.3 shows level of evidence regarding this topic comes mostly from individual studies, with the best level of evidence on Luo et al. in 2011. Latest data available by Chen et al. is a systematic review based on case-control studies. Existing data suggests that hypogonadal subfertile patients will benefit more from varicocele treatment. However, varicocelectomy effects on testosterone levels of fertile patients are still unclear (Table 58.3, evidence level 2b).

Table 58.3 Varicocelectomy in hypogonadic males

Author	Year	Level of evidence	
Hypogonadic males			
Chen et al.	2017	3a	SR of case-control studies
Abdel-Meguid et al.	2014	2b	Individual cohort study
Luo et al.	2011	1b	Individual RCT
Tanrikut et al.	2011	2b	Individual cohort study
Hurtado de Catalfo et al.	2007	2b	Individual cohort study
Sirvent et al.	1990	–	Narrative review

SR systematic review
RCT randomized controlled trials
Levels of evidence based on data from https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/

Subclinical Varicocele Treatment

The benefit of varicocelectomy in patients whose varicocele is impalpable remains debatable. In a study published by Yamamoto et al. in 1996, 85 patients with subclinical varicocele complaining of infertility were randomized to follow-up with no treatment or high ligation of the internal spermatic vein [25]. After 1 year, the authors observed significant improvement in sperm density and total motile sperm count in the surgical treatment group. Pregnancy rates, however, were not statistically different between groups. They concluded that subclinical varicocele surgical treatment results in improvement of sperm parameters, but this does not translate into higher pregnancy rates. In 2001, Unal et al. achieved similar results [26]. In a prospective randomized study, patients with subclinical varicocele were submitted to varicocelectomy or 6 months empirical medical treatment with clomiphene citrate. Both treatments resulted in improvement of semen parameters; no statistical differences between groups, however, were detected. Pregnancy rates were also not statistically different between groups.

A prospective randomized controlled study published in 2018 by Sun et al. investigated the benefits of bilateral varicocelectomy in a subset of patients with right subclinical varicocele [27]. Three hundred fifty-eight patients with left clinical varicocele and right impalpable varicocele were randomized to bilateral or unilateral varicocelectomy. Bilateral surgery resulted in greater improvement in semen parameters than unilateral surgery (sperm concentration, morphology, and progressive motility). No differences were observed in the DNA fragmentation index. Natural pregnancy was also higher in the bilateral treatment group—42.5% versus 26% in the unilateral group.

Finally, in a meta-analysis published in 2018 by Kohn et al., data compiled from 13 studies demonstrated that after subclinical varicocelectomy some improvement of sperm parameters occur—sperm count increases by 13.95×10^6/mL (95% CI 3.23–24.67) and sperm motility by 4.84% (95% CI 4.00–13.67) [28]. Annual pregnancy rate, however, was 11% on the control group and 15% on the varicocelectomy group with a p value of 0.49.

Table 58.4 Subclinical varicocele treatment

Author	Year	Level of evidence	
Subclinical varicocele			
Kohn et al.	2018	3a	SR of case-control studies
Sun et al.	2018	1b	Individual RCT
Unal et al.	2001	1b	Individual RCT
Yamamoto et al.	1996	1b	Individual RCT

SR systematic review
RCT randomized controlled trials
Levels of evidence based on data from https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/

This topic has three individual randomized controlled studies as the best available data to date, as summarized in Table 58.4 (evidence level 1b).

Varicocele Repair in Children and Adolescents

Varicocele, while rare in children, achieves prevalence rates similar to adults in adolescent patients, suggesting that disease onset happens at young age and persists to adult life [29]. Due to its relatively high prevalence in the general population, and since not all varicocele patients experience infertility, hypogonadism, or genital discomfort, the adequate selection of patients that will benefit from treatment is crucial. Treatment decision nowadays usually rely on physical examination (varicocele grade and testicular size) and semen analysis [30]. Nevertheless, ethical concerns, age limitation, and lack of standardized adolescent seminal parameters limit the use of semen analysis in the decision process.

Most studies suggest a benefit on testicular size after varicocele treatment in affected boys. In 1997, Paduch and Niedzielski conducted a prospective study, in which 124 boys with grade 2 or 3 left varicoceles were randomized

to varicocele repair or no treatment [31]. All patients were followed up to 12 months, and treatment choice was the high retroperitoneal approach (Palomo technique). In this study, left testicular growth was higher in the operated patients, achieving volumes similar to the non-affected testicle. Another study published in 1992 by Laven et al. reported smaller preoperative left testis size on boys with varicocele [32]. After 1 year of follow-up, patients submitted to surgical treatment achieved the testis size of the unaffected group, while controls remained with smaller testis.

The repercussion of untreated varicocele on adult life, however, remains uncertain. In 2012 in a Belgian cohort of 661 boys, 372 patients underwent varicocele anterograde sclerotherapy, while 289 were conservatively followed [33]. Average follow-up was 14 years and 13 years, respectively. Paternity rate was not significantly different between groups (85 to 78%, $p > 0.05$). Smaller left testis size in the beginning of the study was also not associated with higher rates of subfertility and paternity at the end of follow-up. In a prospective cohort published in 2017, Chu et al. showed that most adolescents diagnosed with asymptomatic left varicoceles will develop normal sexual development, testicular volume and semen parameters without intervention [34]. This effect was also observed in patients with an initial abnormal semen analysis. Among those patients with abnormal semen analysis, however, 53% will have persistently low total motile sperm count in adult life.

Lastly, a meta-analysis composed of nine RCTs published in 2017 demonstrated an improvement in mean testicular volume by 3.18 mL and in mean sperm count by 25.54 × 10^6/mL following varicocele treatment in boys and adolescents [35].

In conclusion, varicocele repair will most likely improve testicular growth and seminal parameters of affected boys and adolescents. Limitations of the available evidence falls on the absence of RCT with longer follow-ups, and benefits on adult life remain unclear (Table 58.5, evidence level 1a).

Table 58.5 Varicocelectomy in children and adolescents

Author	Year	Level of evidence	
Children and Adolescents			
Chu et al.	2017	2b	Individual cohort study
Locke et al.	2017	1a	SR of RCT
Bogaert et al.	2013	1b	Individual cohort study
Bong et al.	2004	–	Narrative review
Akbay et al.	2000	3c	Ecological
Paduch et al.	1997	1b	Individual RCT
Laven et al.	1992	1b	Individual RCT

SR systematic review
RCT randomized controlled trials
Levels of evidence based on data from https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/

How Varicocelectomy Interferes in Assisted Reproduction Technology Outcomes

The impact of varicocele on ART has also been subject of numerous studies. Some authors advocate that varicocelectomy may improve ART results by promoting improvement of semen parameters sufficient to reduce the complexity of the proposed treatment and/or increasing pregnancy and live birth rates following ART [36–39]. Nonetheless, there are no prospective randomized studies concerning this issue published up to the present time.

Cayan et al. in 2002 proposed that varicocelectomy may improve semen parameters and ultimately lead to a change in the assisted reproduction proposed technology [40]. In this observational study, 540 infertile men with clinical varicocele were submitted to microsurgical varicocelectomy and monitored for the type of ART procedure suggested, based on the sperm parameters. Their results showed that 31% of the patients who would be candidates for intracytoplasmic sperm injection (ICSI) only became candidates for intrauterine insemination (IUI) after surgery. Among in vitro fertilization (IVF) candidates, 53% became candidates to IUI or had the

potential to achieve spontaneous pregnancy. In the same manner, 42% of the IUI candidates became spontaneous pregnancy candidates after varicocele correction.

In 2001, retrospective study published by Ditch et al. showed that varicocele treatment prior to IUI may improve odds of pregnancy and live birth [41]. In this study, 24 men with untreated varicocele and 34 men who had treated varicocele were submitted to IUI cycles. Pregnancy and successful live birth rates were 4.4-fold and 23.6-fold higher in the treated group, respectively.

Some studies also suggest that varicocele treatment prior to ICSI yield better outcomes. In a study conducted by Esteves et al. published in 2010, 242 infertile men with clinical varicocele were submitted to ICSI cycles—80 patients had their varicocele treated prior to ART and 162 patients had their ICSI cycle without treating varicocele [42]. Their results showed better fertilization rates (78.0 ± 20.0 vs. 66.0 ± 22.0, $p = 0.04$), clinical pregnancy rates (60% vs. 45%, $p = 0.04$), and live birth rates (37% vs. 31.4, $p = 0.03$) in the treated group. High-quality embryos, number of embryos transferred, and miscarriage rates were not statistically different between groups. Another retrospective study published by Pasqualotto et al. in 2012, however, achieved contrasting results [43]. In this study, 248 with grade 3 varicocele were submitted to ICSI. At the time of ICSI, clinical varicocele was present in 79 patients, and 169 patients had undergone subinguinal varicocelectomy. No statistical differences were observed between groups regarding pregnancy rates. Preoperative semen analysis, female causes of infertility, and time from varicocelectomy to ICSI, however, were not addressed in the study.

Additionally, Guo et al. in 2013 showed better ART results after varicocelectomy in couples with a previous failure on ICSI [44]. In this study, 49 patients with high-grade varicocele were submitted to microsurgical varicocelectomy. When compared with the previous failed cycle, higher rates of fertilization (72.36% ± 17.88 to 83.36% ± 19.36, $p < 0.05$) and high-quality embryos (34.36% ± 33.27 to 55.67% ± 23.36, $p < 0.01$) were observed. After varicocelectomy, pregnancy rate was 61.2%, including 11 cases of natural conception.

In a meta-analysis published in 2016 by Kirby et al., data compiled from four articles reported non-statistically significant differences in pregnancy rates among oligozoospermic patients submitted to varicocelectomy prior ART (OR 1.695, 95% CI 0.951–3.020, $p = 0.733$) [45]. The meta-analysis from three articles that reported live birth rate, however, showed a significantly higher rate in the group of patients submitted to varicocelectomy (OR 1.699, $p = 0.042$).

In conclusion, considerable data available supports that varicocelectomy may improve live birth rates of ART. The overall quality of evidence, however, is low, with most studies being individual cohorts. Recent data by Esteves et al. and Kirby et al. are systematic reviews (evidence level 1a). Nevertheless, there are no prospective trials available on this subject (Table 58.6).

Table 58.6 Varicocelectomy and assisted reproduction technologies

Author	Year	Level of evidence	
ART Outcomes			
Kohn et al.	2017	–	Narrative review
Samplaski et al.	2017	2b	Individual cohort study
Esteves et al.	2016	2a	SR of cohort studies
Kirby et al.	2016	1a	SR of RCT
Guo et al.	2013	2b	Individual cohort study
Pasqualotto et al.	2012	2b	Individual cohort study
Shiraishi et al.	2012	–	Narrative review
Esteves et al.	2010	2b	Individual cohort study
Çayan et al.	2002	2b	Individual cohort study
Daitch et al.	2001	2b	Individual cohort study

SR systematic review
RCT randomized controlled trials
Levels of evidence based on data from https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/

The Role of Varicocelectomy in Azoospermic Men

Previous reports indicated that varicocele may be present in 4.3 to 13.3% of men with nonobstructive azoospermia (NOA) [46]. Potential benefits of varicocelectomy on those patients are the return of viable sperm to the ejaculate, increasing sperm retrieval rates (SRR) after surgical procedures (microsurgical testicular sperm extraction), and better pregnancy rates, with or without assisted reproductive technology (ART).

The postoperative return of viable sperm to ejaculate is reported in 20.8 to 55% of patients [47]. In the largest cohort of patients to date, published by Abdel-Meguid in 2011, 31 NOA patients were simultaneously submitted to varicocelectomy and testicular biopsy [48]. After 3- to 4-month follow-up, ten patients (32.3%) presented sperm in the ejaculate. Among those patients, mean postoperative sperm concentration was 2.3 ± 1.7 10^6/mL. The only variable that correlated to success was testicular histology—sperm only returned to ejaculate in those patients with histological patterns of hypospermatogenesis or late maturation arrest. Sperm could not be recovered from the ejaculate of patients who presented with early maturation arrest or Sertoli-cell-only patterns. Patient age, infertility duration, varicocele grade, varicocele laterality, FSH, or testicular volumes were not related with success rates. In a meta-analysis published by Esteves et al. in 2016, pooled data from 16 studies resulted in a mean sperm count of 1.82 ± 1.58 × 10^6/mL [47]. There are no controlled studies about this subject.

Three studies have shown benefit of varicocelectomy on patients with NOA regarding SRR [49–51]. In all of them, SRR were higher on patients submitted to varicocelectomy prior to micro-TESE. Pooled data from those studies resulted in an OR of 2.65 (95% CI 1.69–4.14, $p < 0.0001$), favoring the treated patient group [47]. Inci et al. in 2010 also compared ART results after successful sperm retrieval and found no statistically significant differences on high-quality embryos, clinical pregnancy rates, and miscarriage rates between the groups of patients submitted to varicocelectomy prior to micro-TESE [51]. On the other side, Haydardedeoglu et al. reported higher implantation, clinical pregnancy, and live birth rates in the varicocelectomy group [50].

Table 58.7 Varicocelectomy in azoospermic men

Author	Year	Level of evidence	
Azoospermic men			
Esteves et al.	2016	2a	SR of cohort studies
Zampieri et al.	2013	2b	Individual cohort study
Abdel-Meguid	2012	2b	Individual cohort study
Haydardedeoglu et al.	2010	2b	Individual cohort study
Inci et al.	2009	2b	Individual cohort study
Czaplicki et al.	1979	2b	Individual cohort study

SR systematic review
RCT randomized controlled trials
Levels of evidence based on data from https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/

Overall, varicocele treatment in NOA patients may provide viable sperm in the ejaculate and improve SRR. Better pregnancy rates and ART results remain controversial. Urologists face the challenge to adequately select which patients may benefit from the intervention, since this choice of treatment implicates in an additional invasive procedure and longer treatment duration, when compared to sperm retrieval procedure alone. Scientific evidence supporting this comes mostly from individual cohort studies and from one meta-analysis. Level of evidence is summarized in Table 58.7.

Varicocele Treatment Modality

Varicocele can be treated by two main approaches—surgery or percutaneous radiographic embolization. Additionally, varicocele surgery can be performed through various approaches—subinguinal, inguinal, retroperitoneal, and laparoscopic.

A meta-analysis comparing three types of surgical varicocelectomy techniques (high ligation

surgery, inguinal varicocelectomy, and subinguinal varicocelectomy) found no statistically significant differences on sperm count and motility between groups [10].

The meta-analysis published by Agarwal in 2007 divided results from studies in which patients were submitted to high ligation varicocele and microsurgical varicocele [9]. Sperm concentration improved in average by 9.71 million per mL in patients submitted to microsurgical varicocelectomy and by 12.03 million per mL after high ligation varicocelectomy; sperm motility increased by 9.92% and 11.72% in the microsurgical group and high ligation group, respectively. These results, however, were not compared to each other, making it impossible to conclude one technique advantages over another. Additionally, pregnancy rates were not informed.

In 1998, Barbalias et al. prospectively evaluated 88 patients submitted to varicocelectomy, and compared their results regarding the technique used (retroperitoneal, inguinal, subinguinal, and percutaneous venous embolization) [7]. In this study, the subinguinal approach provided greater improvement of sperm parameters, in particular, concerning sperm concentration and motility.

The most common complications associated with varicocelectomy are clinical recurrence, hydrocele, and testicular atrophy. Barbalias reported lowest recurrence rates after the microsurgical approach, versus the highest recurrence following vein embolization and the retroperitoneal approach [7].

Lastly, a meta-analysis published by Cayan et al. in 2013 analyzed results from 36 studies on varicocele treatment [52]. They concluded that radiologic embolization is the technique associated with higher recurrence rate (27%) and requires high skill and experience from the doctor. Laparoscopic varicocelectomy is more invasive than other techniques, can present a high rate of major complications (7.6%), and may not treat the second most frequent cause of varicocele recurrence, the spermatic veins. When comparing open surgery approaches, the authors concluded microsurgical inguinal or subinguinal varicocelectomy yield higher natural pregnancy rates and fewer recurrences and postoperative complications.

Table 58.8 Varicocele treatment modality

Author	Year	Level of evidence	
Treatment modality			
Çayan et al.	2009	2a	SR of cohort studies
Agarwal et al.	2007	1a	SR of RCT
Barbalias et al.	1998	2b	Individual low-quality RCT

SR systematic review
RCT randomized controlled trials
Levels of evidence based on data from https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/

Scientific evidence regarding this topic comes from systematic reviews (Table 58.8, evidence level 1a).

Cost-Effectiveness Analysis

Before the advent of ICSI, varicocelectomy was the only available treatment to infertile men with clinical varicocele. With the popularization of the technology, infertility specialists raised concerns on the cost-effectiveness of adding an additional procedure to an already very expensive treatment.

Schlegel in 1997 published an analysis of cost per delivery concerning the use of ICSI or varicocelectomy as a primary treatment for men with varicocele [53]. The author concluded that varicocelectomy treatment achieved similar live birth rates with lower cost per delivery than ICSI. Another study published in 2002 by Penson et al. achieved similar results [54]. In this study, varicocele effectiveness rates were based on literature published from 1995 to 2000. The costs per live birth were lower on patients submitted to varicocelectomy followed by IVF ($22,114) than on patients submitted to IUI followed by IVF ($22,122) and on patients submitted to IVF immediately ($33,686). Additionally, as mentioned above, varicocelectomy can potentially downgrade the complexity of ART needed in oligospermic patients, reduce the need of sperm retrieval procedures in azoospermic patients, and improve pregnancy rates following IVF, ultimately reducing costs [40, 42, 48] (evidence level 2b, Table 58.9).

Table 58.9 Cost-effectiveness analysis

Author	Year	Level of evidence	
Cost-effectiveness			
Penson et al.	2002	2b	Analysis based on clinically sensible costs or alternatives; limited review(s) of the evidence or single studies
Schlegel et al.	1997	2b	Analysis based on clinically sensible costs or alternatives; limited review(s) of the evidence or single studies

SR systematic review
RCT randomized controlled trials
Levels of evidence based on data from https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/

Conclusion

Varicocele remains the most frequent identifiable cause of male infertility. While the advent of ICSI provided treatment for men who otherwise would not be able to conceive, varicocelectomy still have an important role in reducing the needs of ART and improving results of IVF. Existing data supports the knowledge that varicocele treatment may also improve natural pregnancy and live birth rates, and its treatment should not be overlooked by physicians involved in infertility treatment. Additionally, varicocelectomy may improve testicular pain, increase testosterone levels in hypogonadal men, and preserve testicular function in children and adolescents.

Review Criteria

The current chapter is based on an electronic search using Pubmed/MEDLINE database and references of the identified articles performed between March and May of 2018. The following keywords were used on the search engines: "varicocele," "male infertility," "azoospermia," "testicular pain," and "male hypogonadism."

Multiple Choice Questions and Answers

1. Regarding varicocele treatment, the following statements are true, except:
   (a) Surgical treatment of clinical varicocele may improve semen parameters in infertile men.
   (b) **Subclinical varicocele surgical treatment should be performed to improve pregnancy rates of infertile couples.**
   (c) Men from infertile couples seeking paternity without known female causes should always be evaluated by an experienced urologist.
   (d) Physical examination of the spermatic cords is a prognostic factor for varicocele surgical treatment.
2. Which of the following statement is true about testicular pain?
   (a) Varicocelectomy should be considered in all patients with testicular pain and varicocele diagnosed by ultrasound examination.
   (b) Testicular pain is the most prevalent symptom of varicocele in adults.
   (c) Higher varicocele grade is most certainly related to better outcomes after varicocele surgical treatment.
   (d) **Patients reporting longer testicular pain duration are the ones that benefit most from varicocelectomy.**
3. Varicocele is frequent in infertile couples seeking fertility. Which of the following statements is true on this subject?
   (a) FIV/ICSI is an effective treatment for infertile couples, and varicocelectomy should not be performed prior to the use of this technology.
   (b) **Patients with clinical varicocele submitted to surgical treatment may show better pregnancy rates following assisted reproduction technologies.**
   (c) Women's age should not be taken into account when determining which couples benefit from varicocelectomy prior to FIV/ICSI.
   (d) Sperm DNA fragmentation index does not affect ICSI results; therefore, it should not be used to determine which men will benefit from varicocelectomy.

4. All of the statements below are true, except:
   (a) **Varicocele surgical treatment improves sperm retrieval rates on azoospermic men.**
   (b) Microsurgical varicocelectomy is the only technique capable of improving semen parameters.
   (c) Hypogonadal patients with clinical varicocele should only be treated with testosterone supplementation.
   (d) Varicocelectomy prior to ICSI treatment does not improve positive results.
5. Varicocele onset usually happens in young age. Which of the following items suggests that varicocelectomy should be performed in children and young adults?
   (a) **Unilateral varicocele and asymmetrical testis.**
   (b) Low-grade clinical varicocele.
   (c) Asymptomatic varicocele.
   (d) Clinical varicocele and age less than 15 years old.

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