The Diagnosis and Treatment of Male Infertility

A Case-Based Guide for Clinicians

Nabil Aziz
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Editors

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Foreword

The Editors and Authors are to be congratulated on a timely, comprehensive, and authoritative publication.

Every aspect of the management of male fertility is covered in great detail, and I would suggest that this represents the first comprehensive review of the subject, so thorough that this should be compulsory reading for all clinicians involved in the management of the infertile couple.

Specifically, the authoritative statements about the importance of lifestyle and of anabolic steroid abuse are important and timely. The recognition of the possible advantages of fine needle aspiration (FNA) testicular mapping, combined with micro-TESE (testicular sperm extraction) were timely, and clear statements about the benefits of the treatment of varicocele should be heeded by all who formerly ascribed to erroneous conclusions from questionable meta-analyses.

The implication of the well-reasoned data presented is that investigation and treatment of male infertility might reduce the overall need for assisted reproductive technology (ART) and might also improve outcomes when ART is inevitable.

Even those gynaecologists and specialists in reproductive medicine and endocrinology, who have dedicated their careers to the management of infertile couples by various means of assisted reproductive technology, and who have seen and helped to develop advanced techniques, usually laboratory-based, in the performance of intracytoplasmic sperm injection therapy (ICSI) would concede, after reading this publication, that much can and should be done to treat 50% of the problem, which relates to the male factor.

Too often, urologists are only involved, usually at the request of the patients, after several cycles of ICSI have failed, due to a male factor or to the less helpful and vague diagnosis of unexplained infertility. Increasingly, urologists are seeing cases of secondary infertility, where there has been a similar failure of 1 or 2 cycles of ICSI. Not unreasonably, couples will often ask why there was little attempt to investigate, diagnose and, where appropriate, to treat the male factor. There are, of course, many reasons, some cogent, to ignore the investigation of male factor infertility and to proceed swiftly and efficiently to some form of ART. However, the most frequently given reason is that treatment of male factor infertility "is seldom effective, and that it is unknown whether such treatment positively influences the outcome of ART."

Each chapter in this book explains why these traditionally held views are no longer tenable, and the use of case histories to illustrate effective treatment strategies is thoroughly commendable.

This publication represents timely evidence for the need to investigate and treat the infertile male; it is well presented, well referenced, and authoritative; it is likely to encourage closer collaboration between gynaecologists and urologists and will thus contribute to our common goal.

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Preface

Male infertility is a common problem and is encountered in isolation or in conjunction with female reproductive problems in more than 50% of infertile couples. In spite of this large prevalence, the provision for specialist health care to address a male's reproductive problems remains inadequate compared to his female partner. This anomaly has led to a situation where semen analysis has become the only arbitrator to offer expensive assisted reproductive procedures. Doctors are quick to advise in vitro fertilization and intracytoplasmic sperm injection procedures whenever the results of semen analysis are suboptimal without offering additional tests to identify potential reversible causes for the suboptimal result. Consequently, many men proceed to assisted conception without being fully evaluated even when they have severe but correctable forms of male infertility. However, the field of andrology has been evolving over the past 30 years, which has led to a better characterization of reproductive problems in the male. This has been driven by the belief that men are entitled to know the cause of their reproductive impediment whenever possible. Moreover, the sound clinical diagnosis facilitates medical and surgical therapies specific to these reproductive problems. Even if the treatment to enhance sperm quality does not result in spontaneous pregnancy, the improvement in sperm quality may by itself have a positive impact on the outcome of assisted reproductive techniques. Moreover, the careful diagnosis of the genetic causes of male infertility, such as cystic fibrosis, promotes safer assisted conception.

Our book is organized as a case-based clinical guide to all clinicians involved in managing male reproductive problems, including andrologists, urologists, reproductive endocrinologists, gynaecologists with subspecialist interested in andrology, and their trainees and specialist nurses. We chose the case-based learning approach for the book to support the expanding interest in andrology by presenting the most up-to-date clinical views on assessing and managing fertility impediments in the male. We also feel that ours is a timely book that aids many of us to address the needs of more discerning men wanting to find out the root cause of their infertility and at the same time to meet the demands of health systems driving for more efficient and safe assisted conception technology.

The first two chapters focus on the clinical evaluation of the male fertility potential. The chapter on semen analysis is written in a non-conventional way to drive us away from the mechanistic approach of judging the result, deepening our understanding of the necessity and the limitation of this basic investigative tool. Each subsequent chapter addresses a well-defined clinical problem with pertinent clinical scenarios to promote the clinical relevance of the material presented. We avoid excessive theoretical details to allow enough space for helpful clinical discussion and the sharing of clinical experience in its diversity.

We are grateful to our contributors, who are distinguished leaders in the field with extensive clinical experience from both sides of the Atlantic and the Middle East. The chapters offer us the opportunity to expand our knowledge by comparing our practice and procedures with those from other parts of the world.

This book would not have been possible without the excellent support of Springer International Publishing. We are thankful to Maureen Pierce, Developmental Editor, for her constant support, and Kristopher Spring, Editor at Springer, for managing this project. The editors are grateful to their families for their love and support.

We genuinely hope that this volume will support and enrich your clinical practice in clinical andrology.

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Cleveland, USA Ashok Agarwal, PhD, HCLD (ABB), EMB (ACE)

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Editors and Contributors

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Dr. Nabil Aziz, MBBCH, MD, FRCOG is a Lead Consultant in Gynecology and Reproductive Medicine at Liverpool Women's Hospital and the University of Liverpool, UK.

He received his medical degree from Ain Shams University, Cairo, Egypt. Obtained a doctorate degree (MD) in reproductive medicine from the University of Liverpool, UK in 1999. He became a member of the Royal College of Obstetricians in 1988 and was elevated to a Fellow in 2003. As a Co-Director of the British Andrology Society, he works to promote clinical training in andrology. Dr. Aziz served as an expert at The National Institute for Health and Care Excellence, UK.

He developed the concept of Sperm Deformity Index as a reliable predictor of male infertility. His research work promoted our understanding of the relationship between sperm morphology, sperm apoptosis, and sperm oxidative damage. He has published more than 180 scientific papers, abstracts, and book chapters.

Ashok Agarwal, PhD, HCLD (ABB), ELD (ACE) is the Director of the Andrology Center and Reproductive Tissue Bank, and the Director of Research at the American Center for Reproductive Medicine since 1993. He holds these positions at The Cleveland Clinic Foundation, where he is a Professor at the Lerner College of Medicine of Case Western Reserve University. Ashok received his PhD in 1983 and did his post-doctoral training in Reproductive Biology under a fellowship from The Rockefeller Foundation at Harvard Medical School, Boston. He was an Instructor in Surgery and then an Assistant Professor of Urology at Harvard Medical School from 1988 to 1993. Ashok has over 25 years of experience in directing busy male infertility diagnostic facilities and fertility preservation services. He is very well published and has over 600 scientific papers and reviews in peer-reviewed scientific journals. Ashok 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 32 medical text books/ manuals related to male infertility, ART, fertility preservation, DNA damage and antioxidants. He is active in basic and clinical research and his laboratory has trained over 1,000 scientists, clinicians, graduate and undergraduate students from the United States and abroad. His current research interests include proteomics of male infertility, molecular markers of oxidative stress, DNA integrity, and apoptosis in the pathophysiology of male reproduction, effect of radio frequency radiation on fertility and fertility preservation in patients with cancer.

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Chapter 1
Making a Diagnosis

Ahmad Majzoub and Edmund Sabanegh Jr.

Introduction

Making an accurate diagnosis is essential for optimal male infertility management. Until recently, the assessment of the male had been largely managed by the gynecologist, principally because in vitro fertilization (IVF) is capable of overcoming many male-related semen abnormalities. With the current advancements that are being witnessed in the study of men's health, such a practice, fortunately, is diminishing. The male is responsible for roughly about 50% [1] of the etiology of infertility in couples and thus receiving adequate evaluation and management is as important as female counterpart management. Moreover, a specialized male fertility service would identify and treat a number of conditions that can avoid or ultimately improve assisted reproduction outcomes as well as identify issues that can negatively impact a man's health. Microsurgical reconstructive surgery and sperm retrieval are other examples of the unequivocal need of a specialized male fertility service.

We initiate our discussion by presenting two cases that are commonly seen in clinical practice:

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- **Case 1**: A healthy 32-year-old gentleman presents complaining of failure of conception after 8 months of unprotected intercourse. He denies history of testicular injury, orchitis, undescended testes, or any surgery to the genitourinary system. He has good libido and erectile function. His partner is 27 years of age and has regular cycles and is undergoing evaluation by a female fertility specialist. On physical examination, his body mass index (BMI) is 22 kg/m^2. He has normal secondary sexual characteristics and normal hair distribution. Genital examination reveals a right and left testicular size of 20 ml and 16 ml, respectively. The vasa deferentia are palpable bilaterally and he has a left grade 2 clinical varicocele.
- **Case 2**: A healthy 28-year-old gentleman presents complaining of failure of conception after 14 months of regular unprotected intercourse. He has been evaluated by his primary care physician with a semen analysis that showed a normal volume azoospermia. He denies history of testicular injury, orchitis, undescended testes, or any surgery to the genitourinary system. He does not report erection issues but has been noticing decreased sexual desire. His partner is 27 years of age with irregular cycles and is currently undergoing evaluation for polycystic ovarian syndrome by her gynecologist. On physical examination, he is obese with a BMI of 36 kg/m^2. He has normal secondary sexual characters and normal hair distribution. Genital examination reveals a testicular size of 8 ml bilaterally. The vasa deferentia are palpable bilaterally with no clinically palpable varicocele.

Infertility is classically defined as the inability to conceive after at least 1 year of regular unprotected intercourse. It is a common medical condition affecting between 9 and 25% of couples worldwide [2, 3]. Primary infertility is a term given when no prior conception is attained, while secondary infertility refers to delayed conception in a patient who was previously able to cause a conception. In a systematic analysis of demographic and reproductive health surveys, Mascarenhas et al. reported a 1.9 and 10.5% prevalence of primary and secondary infertility, respectively [4]. Most clinical guidelines agree that a fertility evaluation is generally advisable only after the time frame definition is met. However, couples may be evaluated earlier in the presence of male infertility risk factors such as a history of bilateral cryptorchidism, female infertility risk factors including advanced female age, or when the couple questions the male partner's fertility potential [5]. In addition, we will perform an abbreviated evaluation (focused history and physical exam with semen analysis) in couples prior to 1 year if they express a desire for earlier assessment. As such, the patient in case 1 may be offered evaluation since he is seeking an initial assessment. Nonetheless, a discussion about the statistical chances of pregnancy can be initiated to raise patient awareness and possibly reduce his often experienced anxiety. In a healthy couple, there is about 20% chance of pregnancy with each cycle, provided that no obvious risks for infertility are present [6]. This chance is mainly affected by maternal age and drops to about 5% per cycle when the women approaches 40 years of age. Moreover, about 85% of couples will get pregnant within 1 year of unprotected sexual activity [6].

The Initial Assessment

A detailed reproductive, medical, and surgical history is mandatory during patient evaluation. Details pertaining the couple's sexual encounters are important in order not to miss modifiable causes for their delayed conception. Aspects such as awareness of ovulation time, frequency of intercourse, ejaculation habits, and use of lubricants are examples of important information. The patient's sexual history is also important, particularly his libido and erectile and ejaculatory function. A detailed past medical and surgical history should also be obtained with special emphasis on whether the couple received prior fertility treatment. A checklist of relevant medical conditions must be directly collected such as history of orchitis, sexually transmitted diseases, testicular trauma, undescended testis, and surgeries of the genitourinary tract. Medication history should focus on use of exogenous testosterone, steroids, alpha-blockers, H_2-blockers, anti-neoplastic, and anti-psychotic medications. Moreover, the patient's occupation and environmental exposures with known detrimental effects on fertility are essential. Occupations involving frequent contact with heavy metals (lead, magnesium), pesticides, and solvents can contribute to a decrease in male fertility [7, 8]. Such agents may reduce sperm production, increase the number of defective spermatozoa, and decrease androgen production. Ionizing radiation and exposure to high temperatures can produce similar effects [8, 9]. Normal spermatogenesis requires a temperature that is 2–3 °C lower than that of the body and a temperature rise by 1 °C is capable of reducing the number of spermatozoa by approximately 14% [10]. Workers with excessive heat exposure such as bakers, welders, metallurgists, and cooks are more prone to heat-induced spermatogenesis defects [11]. Finally, a brief review of the female partner's reproductive history may provide crucial information for treatment planning. For example, assisted reproductive technology (ART) may be offered at an earlier stage when a female factor is additionally encountered such as advanced age, endometriosis, or polycystic ovarian syndrome.

The next step is a focused physical examination. The patient's endocrine status can be partly assessed from his general physical appearance. Eunuchoid habitus, reduced facial hair, gynecomastia, and obesity are all presentations that may suggest hypogonadism. Scrotal examination is performed in both the standing and supine positions. It is heralded by inspection to identify scars of prior scrotal or inguinal surgeries and to assess for observable genitourinary conditions such as phimosis, hypospadias, and hydrocele. The testicles are carefully palpated for consistency and masses. Normally, a firm consistency is expected and any change in consistency is indicative of testicular pathology. Small soft testes may signify poor spermatogenesis, while small hard testes may be seen in Klinefelter's syndrome patients. Infertile men are 3 times more likely to develop testicular cancer than the general male population so a careful assessment of testicular consistency is very important [12]. Testicular size can be assessed fairly well with the experienced hands,

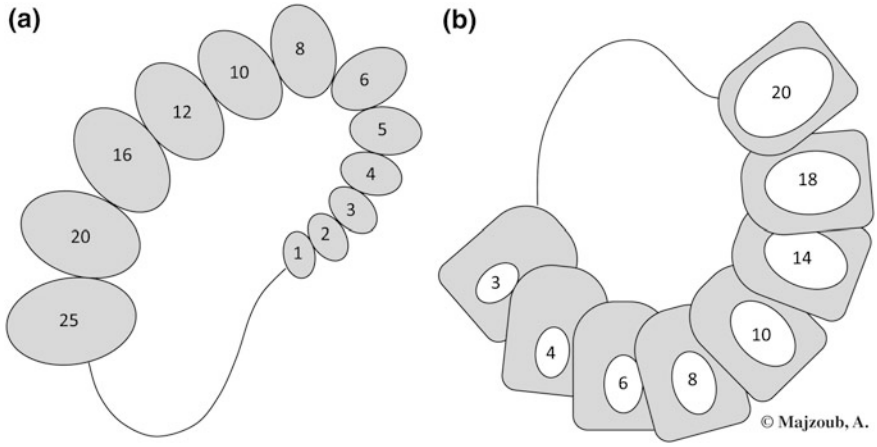

Fig. 1.1 Diagram representing the appearance of **a** Prader and **b** Rochester orchidometers

nevertheless a number of orchidometers or calipers are available. Although Prader [13] or Rochester [14] orchidometers (Fig. 1.1) were found to be inferior to ultrasound in testicular size measurement [15], they still provide an inexpensive and readily available method for size estimation. The epididymes are then assessed for structural abnormalities and degree of fullness. A full epididymis may suggest the presence of obstruction while an underdeveloped epididymal tail is seen in cystic fibrosis (CF) patients or carriers. Palpation of the spermatic cords is then performed to examine the vasa differentia. A number of vasal defects can be identified such as unilateral or bilateral absence of the vas deferens, which is seen in carriers of CF gene mutation. Beading of the vas deferens is also another abnormality that may suggest granulomatous inflammation from tuberculosis infection. The cord is also checked for internal spermatic vein dilatation or varicocele. The Dubin–Amelar grading system is the most common clinical grading system for varicoceles [16]. A grade 1 varicocele is only felt when the patient is asked to do Valsalva maneuver, while a grade 2 varicocele can be palpated without Valsalva maneuver and grade 3 varicocele is visible through the scrotal skin as a "bag of worms." The genital examination is then concluded by palpating the inguinal region for hernia. A digital rectal examination (DRE) may be indicated, especially in those with reduced ejaculatory volume to assess for ejaculatory duct, prostatic, or seminal vesicle pathology. It is also indicated in men aged 50 and above, in the presence of urinary tract symptoms. The size and consistency of the prostate is assessed and masses, cysts, or irregularities are noted. Palpation of seminal vesicles (SV) can occur in ejaculatory duct obstruction (EDO) or in the presence of seminal vesicle pathology.

Semen Analysis

Semen analysis is the initial and single most important test in male fertility evaluation. Although it does evaluate a man's fertility potential, it does not, however, predict with accuracy the likelihood of pregnancy [17]. Patients should be provided with instructions on proper sample collection. Semen analysis should be performed after 3–4 days of sexual abstinence [5], principally because semen volume and sperm count are negatively affected by more frequent ejaculations [18]. In addition, significant reductions in the percentage of sperm motility and normal morphology were detected after 10 days of abstinence [19]. Semen can be collected by means of masturbation into a clean container or by intercourse into special semen collection condoms devoid of substances toxic to sperm. The specimen is preferably collected at the laboratory. If home collection is desired, the specimen should be brought to the laboratory within 1 h of collection and kept at room or body temperature during the transport. To ensure accurate and homogeneous results, samples are better analyzed at laboratories implementing quality control programs for semen analysis and adopting the latest World Health Organization (WHO) protocols for semen testing and reporting of reference values (Table 1.1) [20]. The components of the semen analysis include:

Volume: (Normal >1.5 ml, 5th percentile 95% confidence interval [CI] 1.4–1.7) A small semen volume is most likely due to incomplete collection, but may also be observed in patients with retrograde ejaculation, abnormities of the vas deferens or seminal vesicles, ejaculatory duct obstruction, hypogonadism, and sympathetic dysfunction.

Viscosity: After ejaculation, semen is initially a coagulum that requires 5–25 min to liquefy. Non-liquefaction may occur in ejaculatory duct obstruction or absence of seminal vesicles, while a hyperviscous sample may indicate inadequate secretion of prostatic proteolytic enzymes.

pH: (Normal >7.2) Prostatic and seminal vesicle secretions contribute to the acidity and alkalinity of the seminal fluid, respectively. Ejaculatory duct obstruction may be suspected in patients with an acidic seminal pH and azoospermia. On the other hand, an alkaline pH (>8) measured soon after liquefaction may indicate the presence of prostatic infection.

Table 1.1 2010 World Health Organization (WHO) criteria [20]

Reference values for normal semen parameters	
Semen volume (ml)	≥ 1.5
Sperm concentration (10^6/ml)	≥ 15
Total number (10^6/ejaculate)	≥ 39
Total motility (%)	≥ 40
Progressive motility (%)	≥ 32
Normal forms (%)	≥ 4
Viability (%)	≥ 58
White blood cells (10^6/ml)	<1

Concentration: (Normal 15 million/ml, 5th percentile 95% CI 12–16) Sperm count or the density of sperm reported as millions per milliliter of semen is most commonly detected through counting sperm in a counting grid of a standardized chamber. Oligospermia is a term given when fewer than 15 million sperm/ml are detected, while azoospermia is defined as absence of any measurable sperm in the semen. Azoospermia should further be verified by examining the pellet of the specimen after centrifugation.

Motility: (Normal total motility 40%, 5th percentile CI 38–42) (Normal forward progressive [FP] motility 32%, 5th percentile CI 31–34) Sperm motility is examined after liquefaction at room temperature or preferably at 37 °C. Three classes of motility are typically reported: (1) progressive (PR) motility: space gaining motion; (2) nonprogressive (NP): motion in place or in small circles; and (3) immotility (IM): no motion. Asthenospermia is the term given when a decrease in total motility or forward progressive motility is detected.

Morphology: (Normal forms >4%, 5th percentile, 95% CI 3–4; Kruger's strict criteria—Normal forms >14%) Sperm morphology is routinely examined on a semen smear that has been air-dried, fixed, and stained. The Papanicolauo, Shorr, or Diff-Quik smear stains are advocated by the WHO as they provide adequate color to spermatozoa. Scoring is then performed according to the WHO classification [20], or to Kruger's strict criteria classification [21]. The WHO criteria for normal morphology were originally established after observing spermatozoa recovered from postcoital cervical mucous or from the surface of the zona pellucida. In contrast, Kruger's strict criteria classify sperm as normal only if their shape falls within strictly defined parameters. Regardless of what standard is used, a sperm is considered normal when it has a smooth oval head, intact and slender midpiece, principal piece without breaks, and a clearly visible acrosome with vacuoles not exceeding 20% of the acrosomal area. When less than 4% of sperm have normal morphology, the term teratozoospermia (or teratospermia) is given. A number of defects are identified and classified according to the part of the sperm affected. Head defects include: oval heads, tapering pyriform and vacuolated heads, absence of acrosome (globozoospermia), double heads, and heads with irregular forms (amorphous). Midpiece defects include: thin, thick, or irregular midpiece and asymmetric midpiece insertion into the head. Finally, tail defects include: tail coils, or 90° bends (hammer head), or breaks. Duplication of the tail into two, three, or even four tails on a single sperm is sometimes observed.

Agglutination: (Normal Absent) agglutination is clumping of sperm. The word comes from the Latin *agglutinare* meaning "to glue." It differs from sperm aggregation, which is the adherence of spermatozoa to debris or other elements of the ejaculate. Four grades of agglutination are reported according to the type and extent of interaction.

- Grade 1 (isolated): <10 spermatozoa per agglutinate, many mobile sperm
- Grade 2 (moderate): 10–50 spermatozoa per agglutinate, many mobile sperm
- Grade 3 (strong): agglutinates with >50 spermatozoa, only few mobile spermatozoa

- Grade 4 (complete): completely agglutinated spermatozoa, no mobile spermatozoa visible.

Extensive agglutination is suggestive of immunologic infertility and warrants detection of anti-sperm antibodies.

White Blood Cells: (Normal <1 million/ml) Leukocytospermia is a term given in the presence of WBCs in semen, which can directly or indirectly contribute to infertility [22]. Direct counting of round cells in a semen sample is highly inaccurate because white blood cells can be difficult to distinguish from immature germ cells using light microscopy. As a result, specialized testing needs to be implemented aiming to differentiate between round cells. Immunocytologic staining of semen samples using monoclonal antibodies is considered the gold standard method in this regard [23]. However, it is not widely performed due to difficulties faced in standardizing the monoclonal antibodies used in staining. Consequently, the WHO recommends peroxidase staining as the best next option to diagnose leukocytospermia [20]. Peroxidases are enzymes that break down hydrogen peroxide liberating oxygen, which oxidizes the benzidine derivate present in the staining solution. As a result, a brown color appears that allows the identification of leukocytes under light microscopy. This test works best with polymorphonuclear granulocytes and macrophages as they are rich in peroxidases. However, it fails to stain lymphocytes that represent about 5% of leukocytes in semen [24].

Advanced Semen Studies

Additional sperm tests attempt to identify specific functional deficiencies in sperm. While there is currently insufficient evidence to support the routine use of these tests in patient evaluation, on occasion these tests can be helpful for specific situations [5]. Advanced sperm function testing may be considered in men with unexplained infertility, 1 or more abnormal semen parameters on repeated semen samples, recurrent pregnancy loss or failure of intrauterine insemination (See Chap. 2 for further discussion on level 2 testing). The most commonly performed tests include:

Oxidative Stress

Reactive oxygen species (ROS) are oxygen-containing, chemically reactive molecules that play an important role in cell signaling and homeostasis. The sperm cell produces small amounts of ROS that are beneficial in various sperm functions including promotion of sperm capacitation, regulation of sperm maturation, and enhancement of cellular signaling pathways [25]. Nonetheless, at high levels ROS can be harmful, causing DNA damage, lipid peroxidation, and deactivation of several necessary enzymes [26]. ROS are kept in equilibrium with antioxidants in

the reproductive tract [27]. Antioxidants, which are capable of stabilizing or deactivating free radicals thus mitigating their damaging cellular effects, exist in two forms: the enzymatic and nonenzymatic antioxidant systems. Superoxide dismutase, catalase, and glutathione peroxidase constitute the enzymatic system, while ascorbic acid, urate, tocopherol, pyruvate, glutathione, taurine, and hypotaurine form the nonenzymatic system. When excessive amounts of ROS are produced, or when antioxidant activity fails, this equilibrium state is disrupted, resulting in oxidative stress (Fig. 1.2). Studies have shown that up to 25% of infertile men have significant levels of ROS in their semen, in contrast to low levels in fertile men [28].

Oxidative stress can be directly or indirectly measured. Direct assessment of ROS using electron spin resonance spectroscopy is mainly reserved for research work as it is an expensive procedure requiring great technical expertise [29]. The most commonly utilized indirect technique to measure ROS is chemiluminescence assay. This assay measures the oxidative end products of the interaction between ROS and certain reagents, which results in an emission of light that can be measured with a luminometer.

The clinical value of semen ROS analysis in predicting outcomes with IVF remains unspecified [30]. However, some advantages to ROS testing were proposed. If oxidative stress is identified as an underlying cause of sperm dysfunction, search for lifestyle factors/occupational exposures that may help explain such a finding would be indicated. Therapy with antioxidants would also be a reasonable

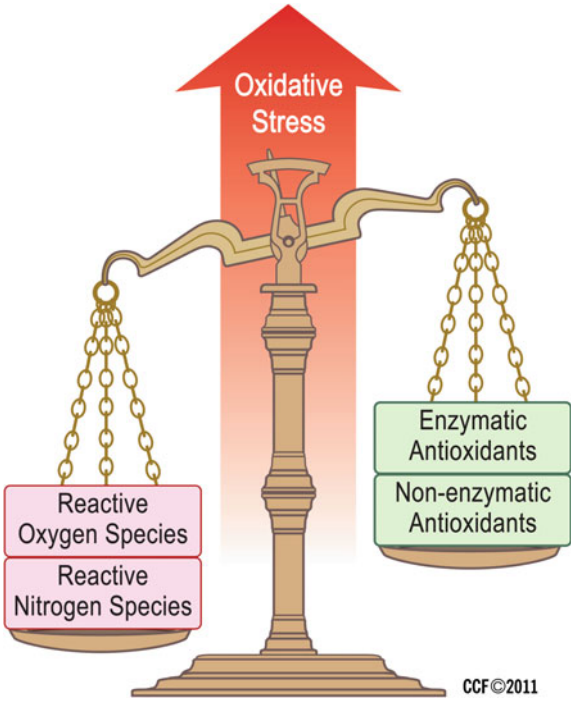

Fig. 1.2 Reactive oxygen species/antioxidant imbalance. © 2011 CCF. Published with permission from Cleveland Clinic Foundation

option attempting to correct the balance between oxidative stress and antioxidant activity. Studies exploring this particular matter generally had mixed results [31].

Sperm DNA Fragmentation

Sperm DNA is bound to protamine and is naturally present in a compact state protecting it from damage during transport [32]. However, damage does exist and at a certain level can be repaired by the oocyte's cytoplasm. But when the damage exceeds the oocyte's threshold, infertility ensues [33]. Sperm DNA damage is believed to affect the couple's fertility through detrimental effects on fertilization, early embryo development, and implantation, as well as pregnancy [34].

Tests of sperm DNA integrity were developed to guide optimal treatment strategies in specific situations. Selection of an assisted reproduction method such as intrauterine insemination (IUI), in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), or performance of varicocele ligation in some instances, are worthy examples. The commonly used methods for detection of sperm DNA damage include sperm chromatin structure assay (SCSA), deoxynucleotidyl transferase-mediated dUTP nick end labeling assay (TUNEL), single cell gel electrophoresis assay (aka, comet), and sperm chromatin dispersion test (SCD) (also-Halo) (Fig. 1.3).

SCSA utilizes fluorescence cell sorting technology to measure the susceptibility of sperm DNA to denaturation when exposed to heat or acids [33]. TUNEL detects "nicks" or free ends of DNA through utilizing fluorescent nucleotides [35]. The comet assay quantifies the actual amount of DNA damage per sperm. The name of the assay comes from the mass of DNA fragments streaming out the head of unbroken DNA, resembling a "heavenly comet" tail [36]. Finally, the SCD measures the absence of DNA damage rather than its presence as following acid denaturation and removal of nuclear proteins, sperm with fragmented DNA fail to produce the characteristic halo of dispersed DNA loops that is observed in sperm with non-fragmented DNA.

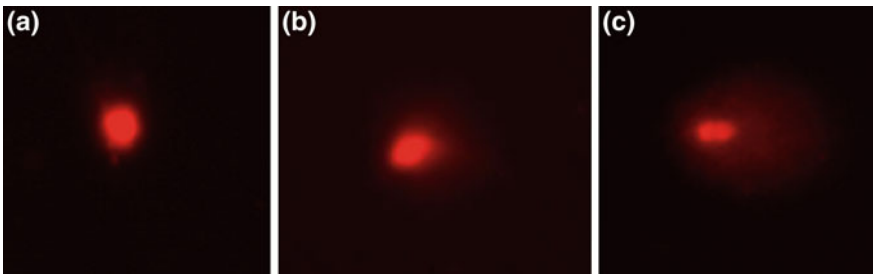

Fig. 1.3 Sperm DNA fragmentation testing using the single cell gel electrophoresis (comet) assay: **a** intact sperm, **b** moderate fragmentation, **c** severe fragmentation

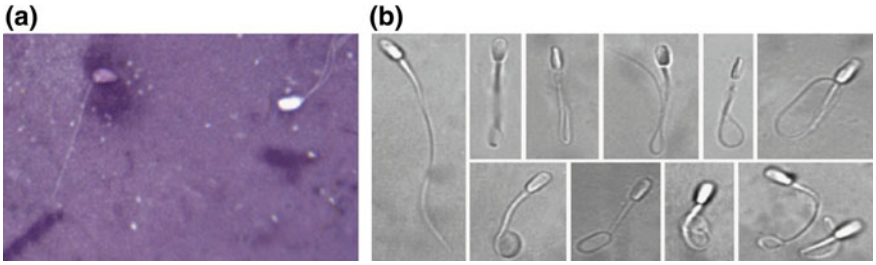

Fig. 1.4 Sperm viability testing. **a** Dye exclusion assays (eosin-nigrosin staining): pink stained sperms are nonviable sperms. Reprinted with permission from Talwar and Hayatnagarkar [66], **b** Hypoosmotic sperm swelling on cat spermatozoa, revealing different grades of swelling. Reprinted with permission from Comercio et al. [67]

Sperm Viability

Viability testing is used to differentiate live from dead sperm in the context of low sperm motility (less than 5–10%) [37]. (Normal is 58%, 5 percentile CI 55–63%.) Another indication is sperm selection for intracytoplasmic sperm injection (ICSI), especially when nonmotile testicular sperm are retrieved surgically [38]. The term "necrospermia" is used when more than 42% of sperm are nonviable. Two methods can be used in viability testing; dye exclusion assays or hypoosmotic sperm swelling (HOS test) (Fig. 1.4).

Dye exclusion assays rely on the ability of live sperm to resist absorption of certain dyes, which can penetrate and stain dead sperm. Examples of such dyes include trypan blue and Eosin Y. A major drawback to this technique is that it requires air-drying after staining, resulting in sperm death and inability to use for ICSI [39].

The HOS is based on the ability of live cells to swell when placed in hypoosmotic media. This test does not damage sperm cells and is therefore utilized for identifying viable sperm for ICSI [37].

Anti-sperm Antibody (ASA) Testing

ASAs are suspected when there is extensive sperm agglutination on semen analysis. Risk factors include prior history of disruption of the blood testes barrier such as that which occurs with genital infection or testicular trauma. Vasal obstruction as well as prior vasovasostomy or vasoepididymostomy can also contribute to ASA development. ASA testing is indicated in the presence of isolated asthenospermia or sperm agglutination and sometimes during the workup of couples with unexplained infertility [5]. Several direct and indirect ASA tests are available such as the mixed agglutination reaction, immunobead test, and immunofluorescence assays.

Post-ejaculatory Urinalysis

Patients with a history of diabetic neuropathy, retroperitoneal surgery, or spinal cord injury may present with dysfunctional ejaculation such as retrograde ejaculation or anejaculation, often presenting as low volume on semen analysis. Other causes of low semen volume include ejaculatory duct obstruction, hypogonadism, or congenital bilateral absence of vas deferens (CBAVD). Post-ejaculatory urinalysis should be performed when the semen volume is less than 1.0 ml, in the presence of a medical history suggestive of ejaculatory dysfunction and in the absence of hypogonadism or CBAVD. Prior to testing, it is important to rule out incomplete collection or short abstinence periods (less than 1 day). After ejaculation, the urine specimen is centrifuged at a minimum of 300× *g* for 10 min. The pellet is then inspected at 400× magnification. Retrograde ejaculation is diagnosed when any number of sperm in a post-ejaculatory urinalysis of a patient with azoospermia is detected. However, in patients with oligospermia, significant numbers of sperm must be identified in urine.

Hormonal Evaluation

The contribution of the hypothalamic-pituitary testicular axis to normal spermatogenesis is well known. As such, an endocrine evaluation is sometimes performed on patients presenting with infertility. Indications for testing include: abnormal semen analysis, specifically when the sperm concentration is less than 10 million/ml; presence of symptoms of hypogonadism; or existence of other clinical findings suggestive of a specific endocrinopathy such as gynecomastia or testicular atrophy [5]. At a minimum, serum follicle-stimulating hormone (FSH) and testosterone levels should be ordered initially and in the presence of abnormal levels, a repeat measurement of total and free testosterone, serum luteinizing hormone (LH), and prolactin levels should be obtained [5]. It is important for the clinician to be aware of the relationship between these hormones to identify the clinical condition. Primary testicular failure is suggested when a low serum testosterone is associated with high FSH and LH levels. Secondary causes of hypogonadism are suggested when both gonadotropins as well as serum testosterone levels are low. A representation of the different hormone findings in various clinical entities is depicted in Table 1.2.

Table 1.2 The hypogonadal pituitary gonadal axis: interpretation of different scenarios

Testosterone	LH	FSH	Prolactin	Clinical condition
—	—	—	—	Normal
↓	↑	↑	—	Primary testicular failure
↓	↓	↓	—	Hypogonadotropic hypogonadism
↓	— or↓	— or ↓	↑	Hyperprolactinemia

— Normal
↑ Increased
↓ Decreased

Genetic Testing

Genetic tests are at times ordered during the workup of patients presenting with infertility. The prevalence of chromosomal abnormalities among infertile men has been estimated between 2 and 7% [40], and is considered significantly higher than the general male population. More importantly, the likelihood of detecting an abnormality in a patient's karyotype is inversely correlated to his sperm count [41]. Indeed, studies have shown that the prevalence of chromosomal abnormalities in patients with azoospermia and severe oligospermia is as high as 35% [42]. Because genetic testing can be expensive, genetic testing should be ordered sparingly, usually in context of moderate to severe oligospermia. We reserve karyotype and Y chromosome microdeletion analysis for patients with a sperm concentration less than 5 million/ml [43]. A number of autosomal and sex chromosomal abnormalities are identified in infertile men. Robertsonian and reciprocal translocations as well as inversions in autosomes were reported in 1.23, 0.82, and 0.13% of infertile French men going for intracytoplasmic sperm injection (ICSI), respectively [44], while sex chromosomal abnormalities were identified in 3.7% of infertile men from the same group [44]. The supernumerary X chromosome seen in Klinefelter syndrome (KS, 47 XXY) is the most common sex chromosome abnormality. Non-mosaic KS accounts for around 11% of azoospermic individuals, whereas mosaic individuals may present with oligozoospermia.

After examining the karyotype of 1170 men, Tiepolo and Zuffardi were the first to hypothesize a correlation between Y chromosome deletions and male infertility [45]. They observed large deletions of the heterochromatic region (Yq12) and the adjacent euchromatic part (Yq11) of the Y chromosome of six azoospermic men [45]. The term "azoospermia factor" (AZF) was later given to this gene or gene cluster and its complexity was only identified after the development of yeast artificial chromosome mapping. Molecular mapping analyses on patients with microdeletions revealed that three nonoverlapping regions of the Y chromosome termed AZFa, AZFb, and AZFc [46] significantly contribute to spermatogenesis and their microdeletion would lead to primary testicular insufficiency characterized by azoospermia or severe oligoospermia. Around 60% of Y chromosome microdeletions occur in region AZFc, while 15% occur in AZFb and 5% in AZFa. The other 20% involve more than one AZF region [47]. AZFa microdeletions carry

the worst fertility prognosis as they are associated with complete absence of germ cells, a condition otherwise known as Sertoli cell only syndrome (SCOS) [48]. AZFb deletions are associated with maturation arrest [48]. Testing for Y chromosome microdeletions is crucial in azoospermic patients before sperm retrieval as the test result carries prognostic information. AZFa, AZFb, or AZFa/b microdeletions are associated with the worst prognosis. Although previously they were thought to be deemed to failure [49, 50], recent studies have reported sperm recovery from few cases of AZFa and b deletions [51]. Whereas patients with AZFc and ABF b/c microdeletions have a surgical sperm retrieval rate of 54.8 and 7.1%, respectively [50]. Moreover, AZFc patients are not necessarily azoospermic, and may be candidates for ICSI using sperm from their ejaculate. It is important in these cases to inform patients that the Y chromosome-bearing sperm will transport the microdeletion to their offspring and that male children born from these patients will also possess similar microdeletions [52].

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene

CF is one of the most common autosomal recessive diseases, occurring in 1 of every 2500 live births [53]. It is caused by a mutation in the CFTR gene, located on chromosome 7 (7q31.2). Patients presenting with congenital unilateral or bilateral absence of the vas deferens (CBAVD) identified during physical examination should be offered CFTR gene analysis, as up to 80% of these men are heterozygotes for CFTR mutations [54]. The most commonly identified mutation is the deletion of a phenylalanine in position 508 (Δ[Delta]508), although more than 1000 different mutations have been identified [55]. Another mutation associated with CBAVD is the 5T allele, which is characterized by the presence of 5 instead of 7 or 9 thymines in intron 8 [55].

Ultrasonography

Scrotal Ultrasonography

Many clinicians routinely perform screening scrotal ultrasonography on fertility patients, largely for screening for occult testicular malignancy or other intrascrotal pathology. In most patients, a proper physical examination by an experienced fertility specialist is sufficient and scrotal ultrasound is not required. Proposed indications for ultrasonography in patients presenting with male infertility include history of undescended or non-palpable testes, prior scrotal surgery, confirmation of a clinical varicocele and when a scrotal or testicular pathology is detected on

physical examination. Non-palpable varicoceles noted on ultrasonography are termed "subclinical" and are not found to be significantly contributing to male infertility [56]. Testicular microlithiasis, which represents the deposition of multiple tiny calcifications throughout both testes, is reported in up to 20% of patients with infertility [57]. Prior concern was raised about its association with testicular cancer, however, current evidence suggests that it is not precancerous and patients may not require more than serial self-examination [58].

Transrectal Ultrasonography

Transrectal ultrasonography (TRUS) is principally considered when the semen is persistently of low volume with or without an acidic pH in patients who are oligo- or azoospermic, with palpable vasa and normal testicular size. Complete or partial ejaculatory duct obstruction is suspected in the presence of dilated seminal vesicles (>1.5 cm in diameter), dilated ejaculatory ducts (>2 mm in diameter), and/or midline prostatic cystic structures on TRUS.

In order to improve the diagnostic accuracy of TRUS in ejaculatory duct obstruction, seminal vesicle aspiration and seminal vesiculography can be performed during the procedure. Jarow was the first to recognize that sperm are not normally present within the seminal vesicles and that EDO should be suspected when 3 motile sperm per high-power field were detected in the SV aspirate immediately after ejaculation [59]. The significance of SV aspiration was evaluated by Eugin et al. in 70 EDO patients. They demonstrated that TRUS alone overestimated the diagnosis as only 49.1% of the patients diagnosed with EDO on TRUS were confirmed with SV aspiration [60]. Seminal vesiculography is another alternative that can be performed during TRUS. After identification of the SV in the axial plane, each SV is punctured with an 18 gauge, 25–30 cm long needle and is injected with water-soluble contrast material. Like SV aspiration, seminal vesiculography improved the efficacy of TRUS in diagnosing EDO [61]. In a study by Purohit et al. [62], only 52% of EDOs suspected with TRUS were confirmed with seminal vesiculography.

Vasography

Vasography has long been considered the gold standard imaging modality for diagnosing an obstructive pathology. However, the invasiveness of the procedure along with the possible risk of iatrogenic vasal obstruction rendered vasography less favorable. Currently, it is at times performed during reconstructive surgery to validate proximal vasal patency [63, 64].

Magnetic Resonance Imaging

Pelvic Imaging

Magnetic resonance imaging (MRI) of the pelvis is sometimes performed during male fertility evaluation to accurately delineate pelvic pathology. It is indicated in the workup of undescended testes, especially when an ectopic abdominal location is suspected, and in the evaluation of the prostate gland, SV, and ejaculatory ducts.

Pituitary Gland Imaging

A number of pituitary abnormalities are known to result in hypogonadism. Sellar and parasellar tumors may disrupt the hypothalamic-pituitary gonadal axis and ultimately affect spermatogenesis. Prolactinomas are the most common pituitary tumors and are divided into micro- (less than 10 mm) and macroadenomas (greater than 10 mm), with larger tumors more commonly associated with clinical manifestations such as headache and visual field changes. Circumstances where pituitary MRI is indicated in the evaluation of male infertility are twofold: increase in serum prolactin levels and/or presence of symptoms suggestive of an intracranial abnormality in a hypogonadal patient [65].

Conclusion

An understanding of different etiologies and risk factors for infertility is mandatory for optimal patient evaluation. A thorough reproductive history together with complete physical examination during the initial encounter is vital. Clinicians should recognize indications for workup selection and should be able to provide sound interpretation of results.

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Chapter 2
Interpreting Semen Analysis and Level 2 Sperm Testing

Nabil Aziz

Introduction

Semen analysis is the first test requested when fertility potential of a man becomes questionable. The results of the semen analysis are often taken as a surrogate measure of his ability to father a pregnancy. The test provides information on the functional status of the seminiferous tubules, epididymis, seminal vesicles, and the prostate. Thus the results of the test should be interpreted in the light of a full clinical history and physical examination of the patient to determine possible causes for reduced fertility potential and of any suboptimal semen analysis result (see Chap. 1).

Although semen analysis is the most practical laboratory assessment of the male we have, it comes with many limitations. The foremost of these limitations is the limited reliability of the result of semen analysis due to variations in the method and timing of obtaining ejaculates and the lack of standardization of the semen analysis methodology [1–4]. There is also limitation of scope as semen analysis is concerned primarily with measuring the volume of the ejaculate and with assessing the number, motility, and the shape of the sperm population. The count of other cellular contents such as spermatids and leucocytes may be noted. The narrow scope of the analysis reduces its ability to identify an underlying pathology when the analysis results are substandard. This in turn contributes to the relatively low prognostic power of semen analysis as a diagnostic test. When azoospermia cases are excluded, the results of semen analyses do not always correlate with pregnancy rates or infertility [5, 6]. Nevertheless, semen analysis provides essential information on the clinical status of the man being investigated.

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Prior to communicating the semen analysis results with patients, the clinician needs to evaluate the significance of the results by answering three questions:

1. Is the semen analysis result reliable?
2. Does it identify a specific pathology?
3. Can this man achieve a pregnancy given his female partner fertility potential?

The Reliability of Semen Analysis Result

There are several factors that may influence the reliability of the semen analysis result. The clinician needs to be aware of these factors before making a clinical judgment on the significance of the result.

Standardization of Semen Analysis Techniques

The standards of semen analysis were first conceived when animal husbandry was being studied in the nineteenth century. The primary focus was on sperm morphology. Sperm count and motility had secondary importance. These same principles were adopted for semen analysis in men in the early twentieth century [7]. This was done without paying much attention to the fact that contrary to other animals, men, as well as chimpanzees, have very high heterogeneity in sperm shape and motility in their ejaculates. Subsequently, several classification systems employing diverse laboratory techniques were proposed to evaluate the quality of semen. This has led to the inability to compare semen analysis results from different laboratories.

The World Health Organization (WHO) addressed this problem by publishing five laboratory manuals consecutively over the past 35 years to provide a standardized approach to the techniques used in collecting and evaluating ejaculated semen. In the first four manuals, cutoff points for each parameter measured were set. The latest fifth manual published in 2010 adopted a new approach by setting lower reference limits derived from examining the semen qualities of 1953 recent fathers [4]. The study population was retrieved from different countries worldwide and with the condition that the time-to-pregnancy was 1 year. The fifth centile (lower reference limit) and its 95% confidence interval (CI) for each semen parameter in these fathers was calculated and used as a guide to judge the quality of other men facing a difficulty in achieving a pregnancy (Table 2.1) [4, 8]. The adoption of these standards worldwide to promote the reliability of semen analysis has been enhanced through a diversity of local internal and external quality control measures including schemes for andrology laboratory accreditation and certification. In spite of this huge endeavor, there remain significant issues with variability of results from different laboratories because WHO methodologies are not always

Table 2.1 Lower reference limits (5th centiles and their 95% confidence intervals, *CI*) for semen characteristics discussed in this chapter

Parameter	WHO 2010 lower reference limits (95% *CI*)	WHO manual (1999) cutoff points
Semen volume (ml)	1.5 (1.4–1.7)	$\geq$ 2 ml or more
Total sperm number (10^6 per ejaculate)	39 (33–46)	$\geq$ 40
Sperm concentration (10^6 per ml)	15 (12–16)	$\geq$ 20
Total motility (PR + NP, %)	40 (38–42)	$\geq$ 50
Progressive motility (PR, %)	32 (31–34)	$\geq$ 25
Sperm morphology (normal forms, %)	4 (3.0–4.0)	Undecided
Vitality (live spermatozoa, %)	58 (55–63)	$\geq$ 50

A complete list of reference limits is found in the WHO manual 2010 [4]. The reference limits are compared to the cutoff points quoted in WHO manual 1999 [8]
WHO—World Health Organization, *CI*—confidence interval, *PR*—progressive motility, *NP*—nonprogressive motility

strictly observed, which may happen intentionally or unwittingly. Moreover, there remains an element of subjectivity in the assessment of sperm count, motility, and morphology with significant inter- and intra-observer variability [9, 10]. Computerized sperm count estimation and motility assessment have been adopted by many laboratories to eliminate the subjectivity of the assessment [11, 12].

Biological Variability

The quality of semen is influenced by many biological variables [13]. These include

- **The completeness of sample collections**: Collecting all the successive emissions (fractions) of ejaculated semen is very important because of the varying composition of the first fraction compared to the latter ones. The first fraction of ejaculated semen is sperm-rich. This is followed by the part of the ejaculate that is diluted by the seminal vesicles fluid [14]. Thus, if the first fraction of the ejaculate is lost, the sperm concentration and total sperm count will be lower than what it should be.
- **The impact of age**: With age both the number of sperm produced and the fluid component contributed by the prostate and seminal vesicles may be reduced [15]. This may give the elusion that sperm concentration in older men is maintained when in reality the total number of sperm in the ejaculate is reduced.
- **Testicular size**: The size of the testis correlates with the total number of spermatozoa in the ejaculate [16]. This is reflective of the level of spermatogenesis in the seminiferous tubules [17]. An extreme clinical example of this association between testicular size and sperm count is encountered in cases of hypogonadotropic hypogonadism and in anabolic steroid abuse cases presenting

with azoospermia and small testicular volume due to suppressed spermatogenesis. The testicular volume increases gradually as spermatogenesis resumes in response to treatment with human chorionic gonadotropin (hCG) injections and abstaining from anabolic steroid use, respectively.

- **Duration of abstinence**: The WHO recommends 2–7 days sexual abstinence prior to semen analysis as a means of standardizing semen analysis. Frequent sexual activity prior to semen analysis may result in reduced semen volume, sperm concentration, and total motile sperm count without significant change in sperm motility and normal morphology [18]. On the other hand, there is significant increase in semen volume, sperm concentration, and total sperm count when the abstinence length is increased [19, 20]. However, length of abstinence does not influence pH, viability, morphology, or total motility [17]. A short abstinence period (24 h) is associated with immature sperm DNA, whereas longer abstinence intervals may be associated with sperm DNA fragmentation [14, 15].
- **The intensity of sexual stimulation**: It has been documented that ejaculates recovered from non-spermicidal condoms used during intercourse at home can be of higher quality than those produced by masturbation and collected into containers in a room close to the laboratory [3]. However, for the sake of standardization it is recommended that ejaculates are collected in a well-equipped room next to the laboratory to reduce the impact of prolonged transport time and the exposure to nonoptimal temperature. The place of producing the ejaculate should be noted in the semen analysis report.
- **Delayed liquidation and viscosity**: Delayed liquefaction and abnormal sample viscosity after complete liquefaction may impact the result negatively. Semen samples are kept at a temperature of 20–37 °C while waiting for sample liquefaction that is normally completed within 30 min of ejaculation. Liquefaction is reported as delayed when it is not complete after an additional 30 min standing. If liquefaction is not achieved after 60 min, mechanical and enzymatic techniques are described in the WHO manual to achieve liquefaction. This should be noted in the analysis report because treatments applied to the sample to achieve liquefaction may affect seminal plasma biochemistry, sperm motility, and sperm morphology [4]. High semen viscosity after complete liquefaction can also interfere with determination of sperm motility, sperm concentration, and the detection of antibody-coated spermatozoa. High viscosity can be recognized by the elastic properties of the sample when a semen droplet is stretched for 2 cm or more between two pipettes. Again the clinician needs to take note of this when interpreting the results.
- **Reversible changes in sperm parameters**: Fever, intercurrent viral infections, medication with drugs such as nitrofurantoin, genital tract infection (epididymitis), and exposure to excessive heat may be associated with reversible deterioration in different semen parameters. When the semen analysis result is suboptimal, these factors should be excluded or corrected before making a final judgment on the quality of semen.

In view of the variability in the quality of ejaculates produced by one man it is impossible to rely on a single semen analysis to characterize a man's semen quality. The analysis of two or more ejaculates may be required to obtain a more valid baseline data to facility a sound view of man's clinical status.

External Technical Factors that May Influence Semen Analysis Result

The result of semen analysis may be impacted negatively by external factors such as the temperature at which the sample is left standing before the start of the analysis and the time elapsed between ejaculation and the start of the test. This information should be noted in the result report before making a considered judgment of its significance.

Does the Traditional Semen Analysis Identify a Specific Pathology?

The semen analysis results should be correlated with relevant medical and lifestyle issues identified during history taking, clinical examination, and other baseline assessments such as hormone profile and scrotal ultrasound scanning. In cases of unexpected azoospermia additional investigations, such as karyotyping and cystic fibrosis gene screen, may be required. Checking for Y chromosome micro-deletions may be sought in severe oligospermia (sperm count $<3 \times 10^6$) on repeated testing. If the analysis reveals teratospermia (sperm with normal morphology <3%) karyotyping is recommended because of the association between teratospermia and chromosomal abnormalities both in somatic cells and spermatozoa [21]. The presence of leucospermia (leucocytic count $>1 \times 10^6$/ml semen) is associated with poor sperm morphology and motility and sperm oxidative stress [22–24].

However, traditional laboratory testing may fail to provide an explanation for the reduced sperm parameters in other cases. This is referred to as idiopathic infertility. Level 2 sperm testing is required in these situations (see later) [25]. But initially let us consider the clinical significance of some of the traditional semen parameter:

The Significance of Semen Volume

The seminal vesicles secretions contribute up to 70% of the normal ejaculate volume. The lower reference limit for semen volume is 1.5 ml (5th percentile, 95% confidence interval 1.4–1.7) [4]. A low sperm volume is more likely to be due to the

incomplete collection of the ejaculate. It also may be due to acquired obstruction of the ejaculatory duct. In cases of congenital bilateral absence of the vas deferens (CBAVD) there is dysplasia or absence of the seminal vesicles resulting in the loss of its contribution to the semen volume [26, 27].

According to WHO standards, retrograde ejaculation should be suspected in any case when the seminal fluid volume is <1 ml [4]. The diagnosis is confirmed by finding spermatozoa in the post-ejaculatory urine sediment when all collected urine is centrifuged and pelleted. The sperm is found mostly dead due to the combined effects of osmotic stress, low pH, and urea toxicity [28]. The recovery of high-quality sperm after the induced modification of the urine composition and pH to facilitate its use in the intracytoplasmic sperm injection technique (ICSI) has been described [29, 30].

Occasionally, the orgasm is associated with a miniscule amount of ejaculate or no ejaculate at all (dry ejaculation, aspermia). This happens in a diversity of neurological diseases and subsequent to surgical procedures on the lower urinary tract [31]. It may also be the presentation of retrograde ejaculation. The explanation for the dry ejaculation is mostly apparent from the initial history taking. If not, then retrograde ejaculation needs to be excluded before referring to the urologist or neurologist for further assessment.

The Significance of Semen pH

The balance between the alkaline secretion of the seminal vesicles and the acidic prostatic secretion determines the semen pH. The importance of assessing the semen pH and its physiological reference range has been a matter of intense debate [32]. However, WHO 2010 sets the lower reference value of the pH of liquefied semen at 7.2 [4]. In CBAVD, the semen pH is characteristically lower (pH 6.8) because of the absence of the seminal vesicles' alkaline secretion. In these cases the scanty seminal plasma is formed mainly from the relative acidic prostatic secretion.

The Value of Semen Analysis in Azoospermic Cases

In the vast majority of azoospermic men, their condition is first identified when they are referred for fertility assessment. In a minority of cases, azoospermia was previously identified including men with cystic fibrosis disease, the majority of Klinefelter's syndrome and, presumably, after vasectomy. Even in these men semen analysis is required to confirm the azoospermic status. Clinically, cryptospermia has been described after vasectomy [33] and up to 10% of men with Klinefelter's syndrome have few sperm in their ejaculates [34–36]. In the andrology laboratory,

azoospermia is suspected when no spermatozoa are observed in replicate wet preparation examined according to the WHO standards [4]. Azoospermia only can be confirmed if no spermatozoa are found in the sediment when the whole ejaculate is centrifuged and pelleted. Cryptospermia describes the absence of sperm in wet preparation but sperm is then found in the sediment. This additional process of semen analysis should be described in the analysis report sent to the clinician. The benefits of a reliable diagnosis of azoospermia in the field of reproductive medicine are multiple. First, an inaccurate diagnosis may lead to an unnecessary invasive procedure to retrieve testicular sperm to treat the couple using their own genetic material. Second, when azoospermia is confirmed additional genetic testing is required to diagnosis the underlying cause. These include karyotyping for chromosomal abnormalities, testing for Y chromosome micro-deletions, and screening for cystic fibrosis gene mutations. Thirdly, the underlying genetic abnormality may have a detrimental impact on the offspring and the affected couples are required to receive adequate genetic counseling prior to undergoing treatment. Finally, the demonstration of absent motile spermatozoa in ejaculate is required after vasectomy to declare the procedure a success.

If azoospermia is secondary to the testosterone hormone or anabolic steroids abuse, periodic repeat semen analyses are required for up to 24 months after abstaining from taking these drugs to monitor the spontaneous resumption of spermatogenesis in many of these patients [37, 38]. If spermatogenesis has not resumed in an acceptable time frame or abstinence is not tolerated, alternative medications may be considered [37].

When considered together, semen volume and pH can help in determining the differential diagnosis of the cause of azoospermia. In patients with low-volume, acidic, azoospermic samples, the differential diagnosis is CBAVD or bilateral complete ejaculatory duct obstruction (EDO). Azoospermic ejaculates with a normal volume and alkaline pH indicate functional seminal vesicles and patent ejaculatory ducts. The differential diagnosis includes spermatogenic failure or an obstruction at the level of the more proximal vas deferens or epididymis, but does not include CBAVD or bilateral EDO.

Can This Man Achieve a Pregnancy Given His Female Partner's Fertility Potential?

In its successive five editions, the WHO manual offered increasingly lower cutoff points to assess sperm parameters. The reference limits for sperm count, motility, and normal morphology quoted in the latest edition [4] are significantly lower compared the cutoff points that were thought to be compatible with normal male fertility in the previous editions. As an example, the cutoff point for sperm count

quoted in the 1999 WHO manual [8] as 20×106^6/mL is almost double the 5th centile of the reference limit (12×10^6/ml) quoted by the 2010 WHO manual [4] (Table 2.1). This may cause confusion among concerned clinicians if the andrology laboratory does not indicate which WHO reference limits values are used. Another reason for the confusion is how the same semen analysis result may be classified as not compatible with normal fertility prior to 2010, but is judged as compatible with normal fertility when applying the 2010 lower reference limits.

At this point it becomes apparent that a semen analysis result is not prescriptive but descriptive of a man's clinical status, with the exception of azoospermia where spontaneous pregnancy cannot happen. This stems from the huge heterogeneity in the characteristics of semen in men compared to other animals. This has led to an extensive overlap of the distributions of semen parameters' results among fertile and non-fertile men (Fig. 2.1c) [39]. This is contrary to the assessment of other biological features where there is distinct distribution or only a minimal overlap of results among affected and unaffected populations (Fig. 2.1a, b) [39]. When that is the case, the identification of a cutoff point to reliably distinguish affected and unaffected individuals is feasible. On the other hand, the extensive overlap in semen analysis results between affected (infertile) and unaffected (fertile) populations causes significant numbers of both false-positive and false-negative cases [39] compromising the prognostic value of semen analysis.

Currently, it is not feasible to define a single useful and reliable "sharp" cutoff level for each individual semen analysis parameter that can distinguish between truly "fertile" and "infertile" men with a high degree of certainty. Although there is a highly significant statistical difference between the two groups, still the degree of the overlap will create low predictive values. For clinicians, the strict scientific interpretation of a reference limit based on "fertile men" is that a result under the limit implies a probability of less than 5% that this sample represents a sample from a "fertile" man. These reference limits, then, should not be referred to as limits of normality. Looking at this from a different perspective, a recent father and a man being investigated for infertility may have a very similar semen analysis result and the only difference may be in the fertility potential of the female partner.

Men with partially reduced fertility are potentially more likely to be infertile when the female also has a reduced fertility potential. In other words suboptimal semen analysis result may be compensated when the female partner has uncompromised fertility potential. This is a clinical judgment that can only be made after full assessment of both partners including clinical history and physical examination.

Level 2 Sperm Testing

From the previous discussion, it becomes clear that restricting semen assessment to the traditional parameters of sperm count, motility, and morphology is associated with numerous concerns. First, for a long time it has been recognized that sperm

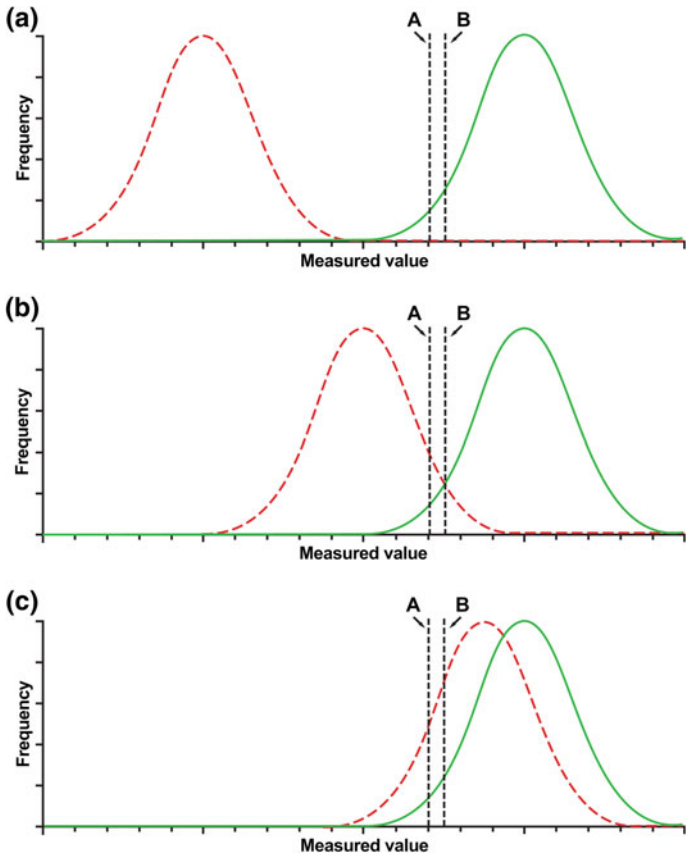

Fig. 2.1 Hypothetical distributions of results from 2 populations: unaffected ("controls") to the right (*green/solid line*) and affected ("patients") to the left (*red/hatched line*). **a** No significant overlapping of results from the 2 groups. A cutoff can be located anywhere between the 2 distributions. If the lower 2.5 percentile (*dotted vertical line A*) of the unaffected population is used as a cutoff, 2.5% of results from the unaffected population will be labeled as "affected." If the lower 5 percentile (*dotted vertical line B*) is used, 5% of the unaffected population will be labeled as "affected." **b** Some overlapping of the 2 distributions. A reasonable cutoff limit would be where the 2 curves cross (corresponding to the lower 5 percentile of the unaffected men, *line B*). Using the lower 2.5 percentile (*line A*) of the unaffected group would label more affected men as unaffected than if the crossing point is used. **c** Gross overlap between results from the groups of men. Using either the 2.5 percentile (*line A*) or the 5 percentile (*line B*) of the unaffected population as a cutoff would lead to most of the affected men being labeled as unaffected. Using the crossing point of the 2 curves would give equal low specificity and low sensitivity so that a large group of unaffected men are labeled as affected. Thus in this case, this parameter is not very useful to distinguish between the 2 groups.

count, motility, and normal morphology may fluctuate, and their assessment can be very subjective and prone to intra-observer and inter-observer variability [40]. Second, although the traditional semen analysis maintains its central role in the assessment of male fertility potential, this often is inadequate to provide a definitive diagnosis of the cause of infertility in many men [41, 42]. Conventional semen analysis cannot cover the diverse array of biological properties that the spermatozoon expresses as a highly specialized cell, such as the presence of sperm apoptosis and chromatin fragmentation [43, 44]. As a result, many infertile couples with no detectable abnormalities are labeled with the clinically convenient but vague diagnosis of unexplained infertility. Third, the predictive power of the cutoff values of the traditional sperm parameters is not absolute, because there is significant degree of overlap in the distribution of the results between fertile and infertile men for each parameter assessed. Fourth, we now have a better understanding of the impact of processes such as sperm capacitation and acrosome reaction [45], sperm oxidative stress (OS) [46], and apoptosis [47] on both sperm–egg interaction and the fertilizing ability of sperm both in vivo and in vitro. The assessment of these aspects of sperm function was included for the first time in the 2010 WHO manual only as research tools [4]. Last but not least, numerous studies in the literature have demonstrated that semen quality is declining and that the incidence of testicular cancers is rising [48, 49]. These observations have been shown to be associated with increased sperm chromatin damage. During in vivo reproduction, natural selection against infertile men limits their opportunity to pass on an infertility trait to offspring, with only rare exceptions. However, some assisted reproduction technologies bypass this natural selection process, leading to the possibility that an abnormal spermatozoon will be selected to fertilize the oocyte.

Sperm oxidative stress, apoptosis, and DNA damage are induced by disease, lifestyle issues, and environmental factors and are implicated in the pathogenesis of male infertility. In view of this current understanding and the availability of the required laboratory additional assessment of sperm damage beyond the traditional sperm parameter of count motility and morphology, this level 2 testing needs to move from the research lab to the mainstream clinical andrology laboratory. When indicated, testing for evidence of sperm OS, apoptosis, and DNA fragmentation can provide a definitive diagnosis of the underlying causes of what is clinically identified as “idiopathic” and “unexplained infertility.” This also may detect men who may perpetually propagate their genetic complement that is linked to male infertility through techniques such as in vitro fertilization using the intracytoplasmic sperm injection (IVF/ICSI). Ultimately, men should be able to find out the exact reason for their inability to father a pregnancy naturally without glossing over the problem by rushing into IVF/ICSI.

It is conceivable that the next task of the WHO is to coordinate the endeavor to determine the most appropriate laboratory tests and the associated standards to achieve this goal. Unless the WHO seeks strict standardization of these tests, the mistakes of the past in assessing sperm count, motility, and morphology will be repeated. The WHO could provide a clinically standardized two-level approach for

semen analysis to achieve a more reliable male fertility assessment. Level 1 will be adequately served by the criteria and standards of the routine semen and sperm parameter to offer an initial screening for men presenting within an infertile relationship. Level 2 testing would have the objective of addressing two different scenarios:

1. To offer a definitive etiological diagnosis for men with abnormal findings in level 1 testing with no clinical explanation (idiopathic infertility); and
2. To seek to clarify if there is an unrecognizable sperm defect on the subcellular level when investigating the male and his female partner did not identify clinical explanation for the couple's fertility (unexplained infertility).

Level 2 testing is also desirable for those who are offered IVF/ICSI. It would appear that the time is ripe for this leap forward in male fertility assessment by expanding the scope of diagnostic features in performing sperm assessment.

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Chapter 3
Medical and Lifestyle Approaches to Improving Semen Quality

Ranjith Ramasamy and Samarpit Rai

Clinical Vignette

Mr. PM, a 35-year-old male and his 32-year-old G0P0 female partner complain of the inability to conceive after 15 months of unprotected intercourse. His past medical and surgical histories are noncontributory. He is an active smoker with a smoking history of one pack per day for 20 years, and he drinks two–three beers every day. His physical examination is unremarkable. The fertility workup of his female partner is normal. His laboratory values are given in Tables 3.1 and 3.2.

Lifestyle Approaches to Improving Semen Quality

There has been a dramatic change in the factors that are potentially associated with alterations in semen quality over the past several decades. For example, there has been a marked increase in the consumption of substances of abuse [1] and the population of smokers [2] worldwide. Centola et al. [3] have demonstrated a significant decline in semen parameters such as sperm concentration (−3.55 million/mL/year, 95% CI −4.87, −2.23; $p < 0.001$), total motility (−1.23%/year, 95% CI −1.65, −0.82; $p < 0.001$), total sperm count (−10.75 million/year, 95% CI −15.95, −5.54; $p < 0.001$), and total motile count (−9.43 million/year, 95% CI −13.14, −5.73; $p < 0.001$) in young adult men over a period of 10 years between

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Table 3.1 Case study laboratory values

Test	Value	Reference range
FSH (mIU/mL)	4	4–10
LH (mIU/mL)	5	4–12
Testosterone[a] (ng/dL)	198	250–1000
Estrogen (pg/mL)	25	10–40
Prolactin (ng/mL)	10	2–18

[a]Drawn on two separate occasions before 10 AM

Table 3.2 Case study semen analysis

Semen analysis (average of 2 separate analyses)		
Concentration	8 million/mL	$\geq$ 15 million/mL
Motility	30%	$\geq$ 50% motility

Table 3.3 Impact of lifestyle factors on seminal parameters

Lifestyle factor	Duration/intensity	Impact on seminal parameters
Smoking	$\geq$ 10 years or $\geq$ 20 cigarettes/day	Impaired sperm DNA integrity, nuclear maturation
Alcohol consumption	$\geq$ 8 alcoholic drinks/week	Impaired spermatogenesis, teratospermia
Exogenous testosterone/anabolic steroids	–	Impaired spermatogenesis

2003 and 2013. This part of the chapter outlines the effects that certain lifestyle factors such as smoking, alcohol consumption, and use of anabolic steroids may have on semen quality and male fertility (Table 3.3).

Smoking

The number of smokers has grown to include nearly one-third of the global population above the age of 15 years [2], despite growing evidence of the deleterious impact of smoking on nearly every organ in the human body. Smoking is associated with male subfertility [2]. Although the exact mechanisms through which smoking impacts male fertility are presently unknown, several theories have been proposed, a couple of which include the reduced delivery of oxygen to the testes that compromises the high metabolic requirements of spermatogenesis, and the oxidative stress conferred by several metabolites of cigarette smoke (tar, benzopyrene, carbon monoxide, aromatic hydrocarbons) [2, 4, 5].

In a large meta-analysis of 57 studies, Li et al. [6] reported that smoking is associated with deterioration of multiple semen parameters such as total sperm count, sperm volume, sperm density, sperm morphology, and progressive sperm motility, both in fertile and infertile men. These results were consistent with the results of another meta-analysis that demonstrated an association between smoking and a reduction in sperm density and sperm motility [7].

In addition to semen parameters, the detrimental impact of smoking on male infertility is compounded by its association with a reduction in seminal zinc (a key antioxidant) levels, sperm vitality, and sperm DNA integrity, and an increase in semen reactive oxygen species. A review of 160 fertile men concluded that fertile smokers showed a significantly higher sperm DNA fragmentation percentage and seminal reactive oxygen species levels, as well as a significantly lower progressive sperm motility, hypo-osmotic swelling test percentage, and seminal zinc levels compared to fertile nonsmokers [8]. Moreover, the quantity and duration of smoking were both positively correlated with sperm DNA fragmentation percentage and seminal reactive oxygen species, and negatively correlated with sperm motility, seminal zinc levels, sperm count, and the percentage of morphologically normal sperm. Furthermore, it has been shown that smoking for more than 10 years or greater than 20 cigarettes/day has a deleterious impact on sperm DNA integrity and nuclear maturation [9]. Sperm fertilizing capacity, as assessed by the zona-free hamster oocyte sperm penetration assay, has also been shown to be markedly reduced in smokers as compared to nonsmokers [10].

A clear understanding of the pathophysiologic mechanisms through which smoking negatively effects semen quality and male fertility notwithstanding, the current scientific literature strongly shows that it significantly impairs male reproductive potential.

In light of his long and heavy smoking history as presented in the aforementioned clinical vignette, Mr. PM should be advised to quit smoking, not just as a critical part of improving his fertility potential, but also because of its deleterious impact on other organ systems such as cardiovascular morbidity and tumorigenesis. He should also be counseled that the detrimental effects of smoking on semen quality have been shown to be reversible with smoking cessation. Santos et al. [11] showed that smoking cessation for 3 months led to an increase in sperm count (72 million vs. 29 million/ejaculate), sperm vitality (20% vs. 60% necrotized), sperm motility (79% vs. 33%), and the number of grade A spermatozoa recovered after swim-up (23 million vs. 3 million/ejaculate).

Alcohol

Alcohol has been shown to have a detrimental impact on male fertility at all levels of the male reproductive system [12]. It compromises the regulation of the hypothalamic–pituitary–testicular (HPT) axis, thereby resulting in a reduction of

luteinizing hormone (LH) and follicle stimulating hormone (FSH) production [13, 14], and the subsequent impairment of spermatogenesis [15].

A study showed that only 12% of the men that consumed alcohol had normozoospermia, as compared to a control group of nonusers in whom the rate of normozoospermia was 37% [16]. Close et al. [17] analyzed the semen parameters of 164 men and concluded that the seminal fluid leukocyte concentration was significantly higher in chronic alcohol users as compared to nonusers. However, after controlling for a past history of sexually transmitted diseases and exposure to multiple substances of abuse, there was only a trend of higher leukocyte count in alcohol users. Consumption of more than eight alcoholic drinks per week has been shown to be associated with male subfertility, with the most common semen parameter anomaly of teratospermia [4]. Additionally, consuming more than 40 grams of alcohol per day showed an increase in spermatogenic disorders [18]. Not only chronic consumption, but even excessive acute alcoholic binges have been shown to be associated with worse semen parameters and an increase in the free estradiol/free testosterone (E2/T) ratio in a cross-sectional study including 347 men [19].

Despite multiple studies investigating the topic, the threshold of quantity at which alcohol consumption negatively affects semen parameters is not well defined. However, it can be inferred from the current scientific literature that consuming more than eight alcoholic drinks per week is detrimental to semen quality [4].

In our clinical vignette, the current average alcohol consumption of Mr. PM is 17–18 drinks—more than twice the upper limit of consumption that is detrimental to male fertility. He should be strongly counseled to reduce his alcohol intake to help restore his fertility.

Exogenous Testosterone and Anabolic Steroids Abuse

There has been a 90% increase in the number of testosterone prescriptions and more than a threefold increase in the use of testosterone replacement therapy (TRT) in men over the past 15 years [20, 21]. In the USA, the rate of TRT has been rising since 2000, but has seen an especially steep increase since 2008 [20]. Furthermore, recent trends suggest that TRT is being prescribed to a significant number of men still in their reproductive years [22].

Exogenous testosterone decreases the levels of FSH, LH, and intratesticular testosterone and impairs spermatogenesis by suppressing the HPT axis [23]. This effect seems to be considerably stronger with intramuscular testosterone as compared to topical formulations [24]. One of the reasons for the high prevalence of testosterone use in infertile men of reproductive age despite its contraceptive effects is the misconception that it enhances fertility—a notion not just limited to patients, but a high percentage of urologists who have indicated that they would use testosterone for the empirical treatment of male infertility [24].

Since it can lead to the atrophy of germinal epithelium, exogenous testosterone may impair spermatogenesis and cause azoospermia within 10 weeks after

initiation of TRT [25]. However, most men are able to regain the baseline spermatogenic function after cessation of TRT [26, 27]. The largest study on the topic that involved men receiving intramuscular TRT for a period of 30 months showed that the median time to recovery for baseline spermatogenesis prior to TRT was 6 months, with more than 99% of the men regaining their baseline spermatogenesis by 15 months [26]. Another integrated analysis of 30 studies evaluated the recovery of spermatogenesis after TRT used as hormonal male contraception, and showed that 90, 96, and 100% of men were producing sperm with a concentration ≥ 20 million/mL by 12 months, 16 months, and 24 months after TRT cessation, respectively [27]. The current scientific evidence clearly shows that most men of reproductive age receiving TRT for treatment of hypogonadism will experience the resolution of their azoospermia or severe oligospermia within 4–12 months after cessation of TRT [28].

Anabolic steroid abuse (without prescription) among men is another significant and underreported issue associated with male infertility, with a lifetime prevalence between 3.0 and 4.2% [1]. Similar to testosterone, anabolic steroids exert a negative feedback effect on the hypothalamus and pituitary glands, thereby suppressing the release of LH and FSH [29] and decreasing the production of endogenous testosterone and spermatogenesis. The deterioration of semen quality after anabolic steroid abuse mainly presents in the form of oligospermia, azoospermia, sperm dysmorphia, and dysmotility [30, 31]. However, most cases of anabolic steroid-induced infertility are also reversible, and usually resolve within 4–12 months after discontinuation of use [32].

Medical Approaches to Improving Semen Quality

Medical treatment is primarily effective in the treatment of secondary testicular failure as opposed to primary spermatogenic failure due to a lack of clear understanding of the multiple discrete defects that lead to idiopathic spermatogenic failure [33]. This part of the chapter outlines the impact that certain hormonal medications such as anti-estrogens, gonadotropins, and aromatase inhibitors may have in the treatment of male infertility, primarily spermatogenic failure (Table 3.4).

Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERMs) such as clomiphene and tamoxifen were the mainstay of treatment for male infertility before the advent of intracytoplasmic sperm injection (ICSI). The mechanism of action of both drugs relies upon their antagonistic activity that blocks the inhibitory feedback exerted by estrogen on the hypothalamus and anterior pituitary, thereby resulting in increased production

Table 3.4 Effects of commonly used medications on seminal parameters

Medication	Effect on seminal parameters
Testosterone	Impaired spermatogenesis
Alpha-blockers	Decreased sperm count and motility
5 alpha-reductase inhibitors	Decreased sperm count in 5% of men
Psychotropic medications	
• Tricyclic antidepressants	Decreased sperm volume and motility
• Lithium	Decreased sperm viability
Anti-hypertensive medications	
• Beta-blockers	Impaired sperm motility
• Diuretics	Decreased sperm concentration and motility
• Calcium channel blockers	Decreased sperm density, motility, and acrosome reaction
Antibiotics	
• Nitrofurantoin	Decreased sperm count at high doses and spermatogenic arrest
• Macrolides	Asthenospermia or sperm death at high doses
• Aminoglycosides	Adversely affect spermatogenesis
Anticancer medications	
• Chemotherapeutic agents	Damage to spermatogonial stem cells and Sertoli cells

Reprinted by permission from Samplaski et al. [52]

of pituitary gonadotropins (FSH and LH) that stimulates spermatogenesis and production of testosterone by testes. Their favorable side effect profile, ease of administration, and low cost makes them viable treatment options.

Unfortunately, the efficacy of anti-estrogens alone has not been established for the treatment of male subfertility since many pertinent clinical trials have used clomiphene and tamoxifen in combination with other agents as the treatment protocol. A randomized controlled trial concluded that in men diagnosed with idiopathic oligoasthenozoospermia, a combination of daily clomiphene (25 mg) and vitamin E (400 mg) led to a significant improvement in total sperm count, forward progressive motility, and unassisted pregnancy rate (37% vs. 13%, $p = 0.04$) when compared to placebo [34]. However, only 30 couples were recruited in each arm of the trial. A meta-analysis of 738 subfertile men with oligoasthenozoospermia that were exposed to short-term anti-estrogen treatment protocols reported only a 2.9% increase in pregnancy rate as compared to the control group (15.4% vs. 12.5%, odds ratio: 1.56; 95% CI 0.99–2.19) [35]. The authors concluded that there is a lack of evidence to support the use of anti-estrogens in the management of oligoasthenozoospermia. In another multi-institutional study by Hussein et al. [36], 64% (27/42) men with non-obstructive azoospermia responded to treatment with clomiphene and produced enough sperm in the ejaculate to be able to undergo ICSI. The dose of clomiphene was titrated to achieve a serum testosterone level between 600 and 800 ng/dL. The posttreatment concentration of sperm ranged between 1 and 16 (mean: 3.8) million/mL. However, the absence of cases of Sertoli-cell-only

syndrome in the studied population and the lack of a control group make it impossible to establish a treatment-related effect of anti-estrogens on fertility outcomes.

Aromatase Inhibitors

Aromatase inhibitors, such as testolactone and anastrozole, increase serum androgen levels by inhibiting the conversion of androgens (testosterone and androstenedione) to estrogens (estradiol and estrone). The mechanism for increased endogenous testosterone relates to the decreased feedback inhibition of estrogens on the pituitary and hypothalamus, leading to greater gonadotropin release [37]. Administration of aromatase inhibitors has been shown to significantly improve semen parameters such as sperm concentration and motility in oligozoospermic men [38]. Most patients that are suitable for therapy with aromatase inhibitors have been shown to have a testosterone-to-epitestosterone (T/E) ratio of <10 [38]. Despite the commercial unavailability of testolactone in the United States, it has been found to be more beneficial than anastrozole for the treatment of subfertile men with Klinefelter syndrome [39].

Gonadotropins

Hypogonadotropic hypogonadism (low FSH, LH, and testosterone) can be idiopathic or have a multitude of other etiologies, such as congenital gonadotropin-releasing hormone (GnRH) deficiency (Kallmann's syndrome), neoplastic (pituitary adenoma, craniopharyngioma, and other central nervous system tumors), or systemic (sarcoidosis, hemochromatosis). Diagnosis and treatment of these underlying conditions with medications, if present, may improve fertility.

Most men with hypogonadotropic hypogonadism benefit from restoration of spermatogenesis with the use of gonadotropin replacement therapy [40]. Although both pulsatile GnRH and gonadotropin therapy appear to be equivalent in improving semen quality and pregnancy rates [41, 42], very few centers use pulsatile GnRH due to the inconvenience caused to patients by having to wear a continuous subcutaneous infusion pump.

The conventional treatment regimen for gonadotropin deficiency involves human chorionic gonadotropin (hCG) as a substitute for LH (1500–2000 IU subcutaneously, twice or thrice per week) with or without recombinant human FSH (rhFSH) (100–150 IU, 2–3 times weekly). The efficacy of this treatment regimen is supported by significant evidence in the literature. In a prospective study, there was an increase in the mean total motile sperm count from zero to 4.8 million, and testicular volume from 4.1 to 12.4 mL [43]. Addition of FSH to hCG is more likely to restore spermatogenesis men with prepubertal onset of hypogonadotropic

hypogonadism, as opposed to men with postpubertal onset in whom hCG alone seems sufficient to produce the desired outcome [44]. Another study of men with hypogonadotropic hypogonadism showed that 81 men achieved normal testosterone concentration but remained azoospermic after pretreatment with hCG, and of these men, 68 (84%) achieved spermatogenesis and 56 (69%) achieved sperm concentration $\geq$15 million/mL after combination therapy with hCG and rhFSH [45]. Another smaller study confirmed the efficacy and tolerance of the combination therapy in 21 azoospermic men with hypogonadotropic hypogonadism [46]. Another multi-institutional, open-label, phase III randomized efficacy and safety study demonstrated that a weekly dose of rhFSH (450 IU), in combination with hCG, successfully induced spermatogenesis in many men with hypogonadotropic azoospermia that initially failed treatment with hCG alone [47]. Time of onset of gonadotropin deficiency and testicular volume are the best predictors of response to gonadotropic therapy [48, 49].

Pulsatile GnRH therapy is typically initiated with a starting dose of 25 ng/kg/pulse administered every 2 h subcutaneously using a portable infusion pump [50]. The dose is adjusted to achieve a mid-normal testosterone level, and a dose of up to 200 ng/kg may be required to induce virilization [50]. The dose can be reduced after the successful appearance of secondary sexual characteristics [50].

There is little evidence to support the use of gonadotropins in men not suffering from hypogonadotropic hypogonadism. In a study that randomized 112 oligoasthenozoospermic men to treatment with 100 U of rhFSH every other day for 3 months versus no treatment, there was no improvement in the seminal parameters of the treated cohort [51]. However, on subgroup analysis, a group of men with cytological evidence of hyperspermatogenesis on fine needle aspiration had a significant improvement in their seminal parameters [51].

Conclusion

This chapter highlights the impact of some of the most common lifestyle and medical factors on sperm quality and male fertility as per existing scientific literature. However, it is very likely that many additional factors that impact sperm quality are yet to be identified and investigated. Further research and well-designed, clinically meaningful studies are required to clearly establish the impact of these factors on sperm quality in order to effectively treat male infertility.

In the case of Mr. PM, he should be strongly counseled to quit smoking and reduce his alcohol consumption. Furthermore, he should undergo comprehensive liver function evaluation, since hepatic disease secondary to chronic alcoholism may cause an increase in serum estrogen levels and affect fertility. His semen analysis should be repeated in 3 months.

Lifestyle areas to consider

1. Occupational hazards (taxi drivers, working in hot environment, use of laptops)
2. Potential impact of cell phone use (microwave)
3. Obesity as a lifestyle issue is widely discussed because of its impact on men's fertility and sexual potency.

Other suggested management approaches

1. Lifestyle modification: clothing, avoiding hot environment, giving up recreational drugs (cannabis, heroin, kata)
2. The role of antioxidants.

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Chapter 4
Testosterone and Anabolic Steroid Abuse

Mohamed Arafa and Ahmad Majzoub

Introduction

Testosterone (T), the male sex hormone, was first extracted by the combined efforts of Ruzicka and Butenandt who were granted the Nobel Prize in Chemistry for their accomplishment [1]. However, this was done almost half a century after the disputed attempt of Harvard Professor Charles Brown-Séquard, who injected himself with "elixir" extracted from the testes of dogs and guinea pigs. Despite reporting improved vitality, strength, and mental facility before the Sociéte de Biologie in 1889, the excessive ridicule and criticism he suffered from his colleagues put an end to his research [2].

Biophysiology of Testosterone

Synthesis

Testosterone is a C19 hormone derived from a C27 cholesterol, predominantly produced from testicular Leydig cells (95%) [3]. The C27 cholesterol molecule passes through several steps of oxidation to produce testosterone. This process

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takes place initially in the mitochondria where cholesterol transport to the inner mitochondrial membrane is facilitated by the steroidogenic acute regulatory protein (StAR) followed by hydroxylation of cholesterol by cytochrome P450 (CYP11A) to C21 pregnenolone [4]. Pregnenolone is then transported to the endoplasmic reticulum where it is metabolized to testosterone through one of two pathways: the Delta-4 pathway or the Δ(Delta)5 pathway (Fig. 4.1) [5].

Transport

Only 2% of testosterone circulates free in the blood while 44% is bound to sex hormone binding globulin (SHBG) and 54% bound to albumin. Testosterone must be unbound to be able to diffuse to cells and perform its action. Since the binding affinity of testosterone to SHBG is 100 times more than albumin, the bioavailable testosterone is comprised of free testosterone and albumin-bound testosterone [6].

Metabolism

Testosterone can be metabolized through three different pathways. The first is aromatization by cytochrome P450 enzyme (CYP19) (aka aromatase enzyme) to produce C18 estradiol. Aromatase enzyme is predominantly expressed by adipose

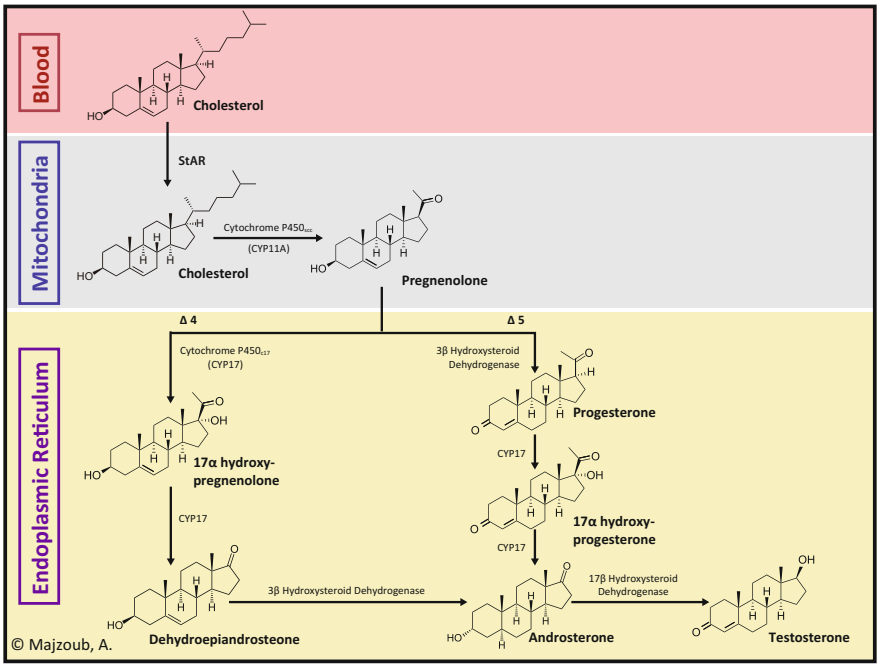

Fig. 4.1 Testosterone synthesis

tissue; however, it is present in small amounts in other tissues including Leydig cells [5]. The second is reduction by 5α(alpha) reductase enzyme to 5α(alpha) dihydrotestosterone (DHT)—an active form of testosterone that binds with much more affinity to testosterone receptors in different body organs. There are two forms of the 5α(alpha) reductase enzyme. Isoenzyme I is expressed by hepatic and somatic cells, while isoenzyme II is expressed in the male genital tract. Finally, testosterone can also undergo degradation in the liver and ultimately be excreted by the kidneys [7].

Regulation of Testosterone Production

Hypothalamic Pituitary Gonadal Axis

Testosterone synthesis is regulated by the hypothalamus and pituitary gland (Fig. 4.2). A neuropeptide hormone called gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus. GnRH then passes through the

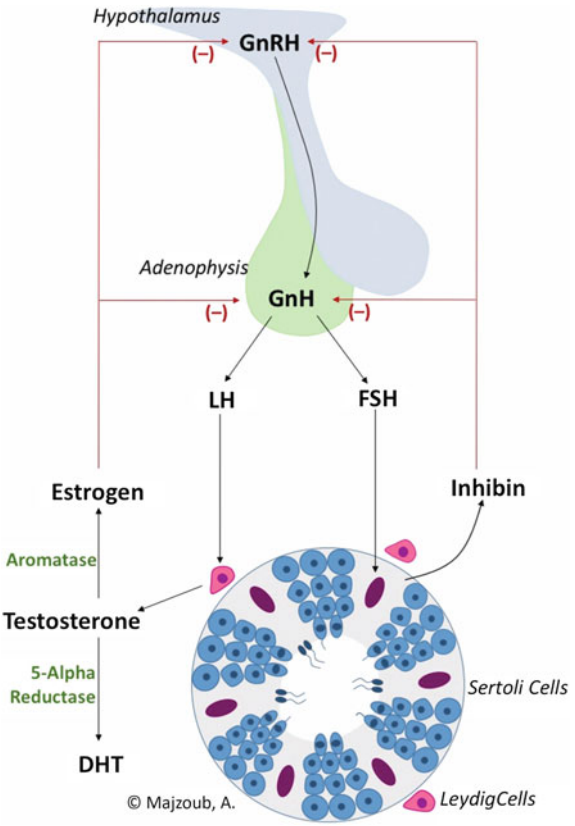

Fig. 4.2 The hypothalamic–pituitary–gonadal axis

hypothalamo-hypophyseal portal circulation to the anterior pituitary where it stimulates the production of the glycoprotein hormones: luteinizing hormone (LH) and follicle stimulating hormone (FSH). FSH and LH are consequently secreted into the circulation to carry stimulatory actions to the testes. FSH acts on Sertoli cells triggering spermatogenesis and hormone synthesis, essentially inhibin. LH on the other hand binds to LH receptors on Leydig cells stimulating steroidogenesis and testosterone production. There is some evidence suggesting that FSH may stimulate testosterone production by Leydig cells secondary to release of activating hormones from Sertoli cells [3]. GnRH is secreted in a pulsatile manner leading to a similar response in LH and consequently testosterone synthesis giving rise to a circadian rhythm that is essential for human health and well being [8].

Testosterone is then aromatized to estradiol, which exerts a negative feedback on the hypothalamus and pituitary gland resulting in decreased production of GnRH, FSH, and LH consequently maintaining testosterone in its optimal range. Inhibin also exerts a negative feedback on the pituitary gland decreasing LH and FSH production [3].

Paracrine Regulation of Testosterone Production

Several nonhormonal factors were proven to regulate testosterone production including insulin-like growth factor 1 and 3, leptin, gherlin, and tumor necrosis factor B. However, their exact effect on testosterone regulation is still not fully established [9].

Physiological Functions of Testosterone

Androgens play a crucial role in the development of male reproductive organs such as the epididymis, vas deferens, seminal vesicles, prostate, and penis. In addition, androgens are necessary for puberty, male fertility, and male sexual function. High levels of intratesticular T, secreted by Leydig cells, are required for spermatogenesis.

Several studies have recognized the effects of testosterone on body composition, including an increase in lean body mass, muscle size, and aerobic capacity [10]. Moreover, supraphysiologic doses of T produce further increments in fat-free mass and strength. The improvement in maximal voluntary strength makes T very appealing among weight lifters, as this phenomenon is critical for superior performance in such events. A positive relationship has been also identified between T and vertical-jumping ability, supporting the idea that T possibly plays a significant role in neuromuscular function [11] or power movements and explaining its use in endurance athletes as well (Fig. 4.3).

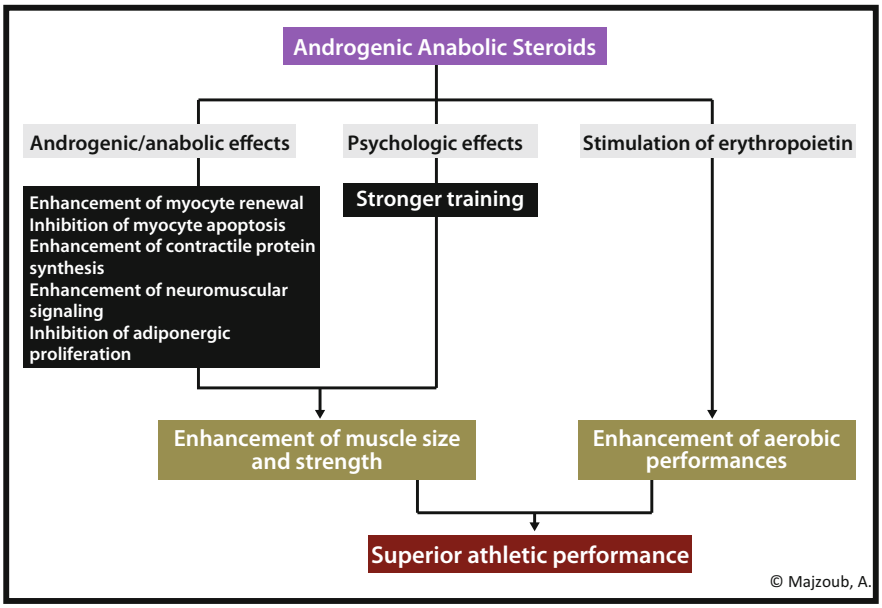

Fig. 4.3 Physiologic effects of androgenic anabolic steroids

What Are the "Anabolic Steroids"?

The study of the anabolic effects of testosterone in the 1930s led to the development of synthetic substances that were named anabolic androgenic steroids (AAS). Their ability to facilitate growth of skeletal muscles was the main reason for their extensive abuse by competitive athletes around the world (Fig. 4.3). In 1974, the International Olympic Committee prohibited the use of AAS by athletes and since then an update of banned substances is issued yearly.

AAS are synthetic metabolites that provide enhanced anabolic effects for their consumer. They are produced from testosterone, which is chemically modified to prevent its rapid metabolism by the liver, increase its half-life and subsequently its overall anabolic action. This was initially done by alkylation of the molecules but this resulted in major liver toxicity, so newer modifications were used including methylation, chlorination, or aromatization. All these chemical modifications result in the production of AAS with high concentration, longer half-life and more potent anabolic effect than the original testosterone molecule. Table 4.1 includes a list of various AAS available in market [12].

Table 4.1 Androgenic anabolic steroid list and doses

Androgenic anabolic steroids	
Generic name	Dosage (mg)
Oral agents	
Fluoxymesterone	10–40
Metyltestosterone	10–25
Oxandrolone	10–20
Oxymetholone	50
Stanozolol	5–15
Ethylestrenol	10–20
Metyltestosterone	5–10
Methenolone acetate	25–50
Quinbolone	20–40
Norethandrolone	10–20
Mesterolone	25–75
Testosterone undecanoate	40–160
Injectable agents	
Nandrolone (different preparations)	50–100
Trenbolone	76–152
Testosterone (different preparations)	100–200
Dromostanolone	100
Methenolone enanthate	100
Methylandrostenediol	50–100
Oxabolone	25–50

Testosterone and Anabolic Steroids Abuse

Nonmedical (Athletic) AAS Abuse

Clinical Case Scenario 1

A 29-year-old man presented to the clinic complaining of primary infertility of 1 year duration. For the past 6 years, he has been engaged in serious bodybuilding exercises and was advised by his trainer to receive testosterone injections to enhance his muscle mass and power. He received testosterone enanthate 250 IU intramuscular (IM) injection every other day for 1 month—a course that was repeated 4 times a year for 3 years. His last course dated 2 years back. He complained of a decreased libido but his erections were normal.

General examination revealed a muscular patient with normal body mass index and normal secondary sexual characteristics. Both testes were in the scrotum with normal size and consistency during local genital examination, without any palpable abnormalities in the epididymis, vasa deferentia, and spermatic cords. His semen analysis showed azoosepermia and his blood test results revealed low serum testosterone.

This case is a classical representation of AAS abuse frequently encountered around the world. In 2004, the medical commission of the International Olympic Committee (IOC) reported that around 1% of athletes tested positive for AAS during the last decade. However the true prevalence of AAS abuse is anticipated to be much higher [13], especially abuse in noncompetitive athletes that is not recorded. Self-report surveys from adolescents showed a prevalence of AAS abuse ranging from 1–6% [14–16]. The most frightening fact was that this abuse started as early as the age of 15 years.

Although hormones are strictly purchased with a prescription, the black market represents a major threat to the medical safety of individuals by providing them to athletes without any control. Actually, trainers deliver AAS to athletes with the intention of producing massive and rapid effects on muscle bulk in a very short time —a process that cannot be attained even with multiple diet regimens and regular exercises. The profile of AAS abusers has changed with time. The media that defined the attractive man model as being muscular and a strong "Superman" probably influences this change.

Although the prevalence of AAS abuse is very high, its actual benefit in sports is still debatable. Some studies concluded that AAS had no effect on muscle mass or performance while others strongly encourage its use based on significant positive effects on athletes' performance [17, 18].

The Therapeutic Use of Testosterone

Testosterone replacement can be considered in patients experiencing symptoms of hypogonadism along with biochemical evidence of low serum testosterone levels. It is typically prescribed for patients complaining of decreased libido, erectile dysfunction, fatigue, decreased muscle mass, depression, lack of concentration, and a low sense of wellbeing.

Clinical Case Scenario 2

A 46-year-old banker presents with secondary infertility for 5 years. Four years earlier, he consulted a urologist to complain of decreased libido. The urologist started him on long-acting testosterone after blood testing revealed low serum testosterone. Unfortunately, this is a commonly encountered incident in andrology clinics, where some doctors abuse testosterone for treating sexual dysfunction without counseling the patient regarding its side effect.

Effects of Testosterone and Anabolic Steroid Abuse

Effects on Male Fertility

Exogenous administration of testosterone or its synthesis derivatives induces feedback inhibition on the hypothalamic–pituitary axis resulting in reduction of FSH and LH synthesis and consequently a decrease of intratesticular T levels. Infertility after AAS abuse commonly presents as oligozoospermia or azoospermia, associated with abnormalities in sperm motility and morphology [19].

Histopathologic evaluation on testicular tissue in AAS abusers revealed Leydig cell alterations mainly [20]. Moreover, impairment of spermatogenesis with a picture of maturation arrest has been described [21]. After AAS discontinuation, Leydig cells tend to proliferate but remain below the regular counts, even after longer periods. Clearly, long-lasting, or possibly persistent effects of AAS use cannot be ruled out. Apoptosis has been reported to play an important role in the regulation of germ cell populations in the adult testis. Recently, the correlation between apoptosis and high AAS doses and exercise has been experimentally assessed in animal models. Shokri et al. [22] report a significant increase in the rate of apoptosis of spermatogenic cells after nandrolone administration—an increase clearly amplified by physical exercise.

Effect on Sexual Function

Erectile dysfunction (ED) is common in patients receiving AAS. It usually starts 5–6 weeks after the initiation of treatment and is most notable with nandrolone and trenbolone. Possible reasons for this consequence include excessive estrogen levels or reduction of DHT levels. Estrogen is commonly elevated secondary to unopposed aromatization of exogenous T. Kwan et al. [23] confirmed existence of inhibited sexual activity, spontaneous erections, and nocturnal penile tumescence in men receiving estrogen. In addition to its negative feedback effects on gonadotropin secretion by the hypothalamus and pituitary gland, estrogen competes with testosterone on binding to ARs throughout the body. As estrogen levels increase, testosterone cell stimulation may be locked in the "off" position, thus reducing sexual arousal and causing loss of libido. DHT is essential for erectile function. Again, inhibition of the hypothalamo–pituitary–gonadal axis results in reduction of endogenous T and consequently DHT causing ED.

Other Effects of Testosterone and Anabolic Steroid Abuse

Cardiovascular Effects

Several reports have linked AAS abuse to cardiovascular morbidity and even mortality [24]. These dreadful events tend to occur in young men without any previous cardiac history, and are secondary to atherogenic changes, thrombogenic effects, vasospasm effects, and direct myocardial toxicity that is often seen in AAS abusers [25]. Autopsy studies revealed the presence of hypercontracted, deeply eosinophilic cardiac myocytes [24], likely representing exposure to increased sympathetic activity. Certainly, this exaggeration of sympathetic activity has been found in few animal studies to be triggered by androgens [26]. AAS abuse results in left ventricular (LV) concentric hypertrophy that seems to persist years after discontinuation [27]. Atrial fibrillation, ventricular arrhythmia, diastolic dysfunction, and sudden cardiac death are all linked with this concentric remodeling of LV hypertrophy. Recent echocardiographic studies showed that both systolic and diastolic dysfunctions were directly proportional to the dose and duration of AAS use [28]. The utilization of AAS has been associated with polycythemia and adverse alterations in clotting factors [29]. A few reports have demonstrated that nonaromatizable androgens, such as stanozolol, can reduce plasma high-density lipoprotein by more than 30% [30]. AAS additionally increase hepatic lipase activity, subsequently worsening the dyslipidemia. All these factors place AAS abusers in real danger of life-changing cardiovascular incidents.

Hepatic

AAS use has been associated with elevations of various liver functions tests such as alkaline phosphatase, aminotransferases, conjugated bilirubin, and plasma proteins [31]. In the majority of cases, this elevation of liver enzymes is transient, with levels normalizing about 2 weeks after sensation of AAS use. Jaundice has been reported to occur following 2–5 months of treatment. Moreover, 17-alpha alkylated agents were particularly associated with cholestasis [32]. Breakdown of skeletal muscles amid extreme training may bring about an elevation of transaminases and hence should be assessed with caution in competitive athletes. Peliosis hepatis and hepatocellular adenomas have been also seen with the utilization of 17-alpha alkylated AAS [33]. Strong evidence linking hepatocellular carcinoma with AAS is lacking [34].

Musculoskeletal

Estrogen produced by excessive aromatization of testosterone may result in premature epiphyseal plate closure. Androgens can have a paradoxical catabolic effect on tendons and ligaments characterized by an increased risk of rupture of biceps and quadriceps tendons [35]. Ultrastructural analysis of tendons in rodents treated with anabolic steroids shows dysplasia of collagen fibrils [36].

Subcutaneous Tissue

Acne fulminans and acne conglobate are the two most common forms of acne seen in almost half of AAS abusers [37]. Sebaceous gland hypertrophy, cysts, increased skin surface fatty acids, and increased cutaneous populations of propionibacterium acnes are all contributing factors to acne formation in this population. Anecdotal evidence shows that acne associated with AAS can get worse with vitamin B supplements [37]; hence, subjects using AAS should be warned about this consequence.

Balding is another consequence resulting from continued steroid use. It is believed to be secondary to androgen receptor-mediated gradual transformation of active large scalp epithelial hair follicles into smaller dermal villus follicules [38].

Neuropsychiatric

Several case reports of adverse central nervous system effects for AAS have been described. Negative feedback inhibition of the pituitary gland may result in secondary empty sella syndrome [38]. Persistent hiccups were also reported in an elite bodybuilder abusing AAS, suggesting a brain stem locus of involvement [38]. Permanent central vertigo and insomnia are other symptoms that have been linked to AAS abuse [38].

AAS use has been associated with a variety of psychiatric disturbances such as aggression, dysthymia, psychosis, and criminal behavior. The severity of these symptoms appears to be dose-dependent. Twenty-three percent of medium- and high-dose abusers (more than 300 mg AAS/week) did meet the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R) criteria for a major mood syndrome (mania, hypomania, and major depression) and up to 12% of them developed psychotic symptoms [39]. In a double-blind, randomized, placebo-controlled crossover study, eight men received increasing doses of testosterone cypionate (150 mg/week for 2 weeks, 300 mg/week for 2 weeks, and 600 mg/week for 2 weeks). The subjects' aggressive responses were experimentally evaluated and were found to be significantly higher among patients receiving

supraphysiologic doses of T [40]. Another placebo-controlled study showed that manic symptoms are more prevalent among men receiving 600 mg per week testosterone cypionate than men receiving lower doses or placebo [41]. Moreover, delusions of grandiosity, elation, criminal behavior, and acute confusional states has also been associated with AAS abuse [42]. Interestingly, AAS abusers exhibited dependency on other substances with approximately 70% of them meeting criteria for alcohol dependence [43], followed by opioid dependence [44].

Diagnosis of Testosterone and Anabolic Steroid Abuse

Patients abusing testosterone or anabolic steroids are commonly encountered in male fertility clinics because of inability to father a pregnancy. The diagnosis of AAS abuse is based on a number of phenotypic, physical, and laboratory characteristics. The majority of patients doping on AAS appear "buffed-up." Physical examination may reveal acne on the back or face, premature baldness, striae or stretch marks especially on the axillae, gynecomastia, signs of heart failure, hepatomegaly, and testicular atrophy. Laboratory investigations reveal normal volume azoospermia or oligospermia on semen analysis, low serum FSH and LH secondary to feedback inhibition, variable serum testosterone level depending on the type and dosage of the abused substance and the timing of the blood test, and a variable serum estradiol level depending on the type of the abused substance and on whether stacking with aromatase inhibitors is performed.

AAS detection is principally executed by various sport authorities fighting against their use in competitive sports. The 1954 World Weightlifting Championships witnessed the first reported use of AAS by competitive athletes [45]. Testing, however, started more than 20 years later at the 1976 Olympic Games in Montreal and was mainly based at that time on radioimmunoassay techniques [45]. During recent decades, the number and forms of doping substances substantially grew, calling for a proportional advancement in diagnostic methods. Of all doping substances in 2012 reported by the World Anti-Doping Agency (WADA), about 50% were anabolic steroids [46]. Urine, blood, hair, saliva, sweat, and nails can all be used as biological specimens to perform laboratory drug testing, each providing different levels of specificity, sensitivity, and accuracy. Urine is mainly the preferred test substance as it is easy to collect. Concentrations of drugs and drug metabolites also tend to be high in the urine, allowing longer detection times than in other body fluids. Several methods have been implied in the detection of AAS, they include gas and liquid chromatography, mass spectrometry, enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays, urinary testosterone-to-epitestosterone (T/E) ratio, and measures of the biologic activity of endogenous androgens.

Management of Testosterone and Anabolic Steroid Abuse

Male Infertility Management

Cessation of AAS abuse is fundamental in the treatment of patients seeking fertility. Patients should be encouraged that recovery of spermatogenesis after drug discontinuation is generally promising. A World Health Organization (WHO) Task Force study evaluated the effect of testosterone enanthate on semen parameters of 271 men [47]. After 6 months of treatment, azoospermia was detected within a mean duration of 120 days in 65% of men. Recovery was documented in 84% of men who had a sperm density >20 million/mL after a median of 3.7 months [47]. This study and few others demonstrated recovery of spermatogenesis in patients receiving testosterone as a method of contraception. The situation may not be similar to what is encountered in clinical practice. AAS abusers generally receive supraphysiologic doses of steroids and perform stacking with multiple formulations, resulting in effects that may be more detrimental on testicular function and degree of recovery of spermatogenesis.

A major disadvantage for abrupt cessation of AAS use is that patients often suffer from the resulting state of hypogonadism that occurs. Recovery of the hypothalamic–pituitary–gonadal axis is usually gradual, meaning that patients often develop symptoms of hypogonadism for a period of time until recovery ensues. Aiming to enhance testicular endocrine function and spermatogenesis, many clinicians resort to chemically increase endogenous testosterone productions through the use of selective estrogen receptor modulators, aromatase inhibitors, and gonadotropin analogues.

Selective Estrogen Receptor Modulators

Clomiphene citrate (CC) and tamoxifen are selective estrogen receptor modulators (SERM) that competitively bind to estrogen receptors on the hypothalamus and pituitary gland. As a result, they prevent the inhibitory effect of estrogen on gonadotropin production thereby increasing LH and ultimately testosterone production by the testes. Potential side effects include facial flushing, excessive sweating, gynecomastia and breast tenderness, weight gain, hypertension, cataracts, and acne.

The efficacy of CC in increasing serum T is well established. Taylor and Levine compared CC efficacy to T gel replacement therapy [48], with an average post-treatment testosterone of 573 and 553 ng/dL in the CC and T gel groups, respectively. CC is an established alternative treatment for men with hypogonadism, demonstrating biochemical and clinical efficacy with few side effects and lower costs than T replacement. A recent investigation from Memorial Sloan-Kettering Cancer Center verified the safety and efficacy of long-term CC use [49]. Eighty-six men with hypogonadism were treated with 25–50 mg CC for a mean duration of

19 months resulting in an average testosterone level of 550 ng/dL with significant improvements in libido and energy [49].

Existing studies on the influence of CC and tamoxifen on sperm production confirm favorable outcomes. Patankar et al. [50] treated patients with oligospermia (including severe oligospermia) with only 3 months of CC and demonstrated a statistically significant improvement in semen parameters. A recent meta-analysis of 11 randomized controlled trials found that anti-estrogen therapy was associated with a statistically significant increase in sperm concentration (weighted mean difference [WMD] 5.24; 95% CI, 2.12–88.37; $p = 0.001$), and sperm motility (WMD, 4.55; 95% CI, 0.73–8.37; $p = 0.03$) [51]. Moreover, the authors concluded that treatment with CC or tamoxifen for 3–6 months resulted in a statistically significant increment in pregnancy rate.

Human Chorionic Gonadotropin

Human chorionic gonadotropin (hCG) is an LH analog administered subcutaneously or intramuscularly 2–3 times per week at doses of 2000–3000 units. It stimulates Leydig cell production of endogenous T and is capable of initiating spermatogenesis in men with hypogonadotropic hypogonadism [52]. This effect, however, occurs for a short duration necessitating the use of FSH to maintain improvements in spermatogenesis. In one case series, 13 azoospermic men with hypogonadotropic hypogonadism were initially treated with a combination of hCG and human menopausal gonadotropin (hMG) to stimulate spermatogenesis; hMG was then withdrawn and patients continued treatment with hCG alone for a period ranging from 3 to 24 months. After 12 months of treatment, sperm counts significantly decreased in all patients with one of them becoming azoospermic [53].

Human chorionic gonadotropin administered in lower doses has also maintained a favorable testicular response. In a randomized trial by Roth et al., experimental gonadotropin deficiency was induced in 37 normal men with GnRH antagonists. Patients were then randomized to receive either several low doses of hCG or T gel daily. Testicular fluid obtained by percutaneous aspiration was assessed at baseline and after 10 days of treatment. Results indicate that intratesticular T levels increased in patients receiving far lower doses of hCG than what is therapeutically prescribed [54].

Human Chorionic Gonadotropin and Testosterone

Human chorionic gonadotropin has been utilized in clinical practice in combination with exogenous testosterone specifically in patients experiencing detrimental effects on their wellbeing after stopping testosterone replacement.

Aromatase Inhibitors (Anastrozole and Letrozole)

Aromatase is an enzyme that belongs to the cytochrome P450. It is present in the testis, liver, brain, and adipose tissue and is responsible for converting T to estradiol. Aromatase inhibitors block this conversion thereby minimizing the negative effects of estradiol on gonadotropin secretion and intratesticular T production. The subsequent increase in levels of LH, FSH, and testosterone consequently improved spermatogenesis [55]. Such medications are most commonly indicated in the treatment of male infertility in obese patients or when the testosterone-to-estradiol ratio is less than 10.

Aromatase inhibitors have been mostly evaluated in the management of age-related hypogonadism. In a recent study by Dias et al. [56], aromatase inhibitors consistently increased serum T levels and, more importantly, improved patients' bone mineral density. However, in a randomized, double-blind, placebo-controlled trial, anastrozole resulted in an inferior elevation in serum T level than CC [57].

Sexual Dysfunction Management

Sexual dysfunction is commonly encountered in AAS abusers after quitting steroid use or even while doping. Reasons include the body's response to specific steroids, estrogen imbalance, testosterone deficiency, and 5-AR inhibitor use. Nandrolones, for example, are very similar to synthetic progestins having central nervous system depressing effects on sexual function. AAS at doses comparable to human abuse levels reversibly blocked sexual receptivity and interrupted neuroendocrine axis in rats [58].

Management of sexual dysfunction in AAS abusers should start with correction of the underlying hormone imbalance as stated previously. Oral phosphodiesterase inhibitors (PDE5i) can be used to bridge the period until hormonal imbalance is corrected. An inferior response to PDE5is can be expected while hypogonadism is prevalent. Indeed, Yassin et al. [59] demonstrated that correction of hypogonadism is pivotal in the management of erectile dysfunction in hypogonadal men. Another study compared PDE5i monotherapy versus PDE5i and T in hypogonadal men and reported a superior outcome on erectile function with the combination therapy [60]. Patients not responding to medical treatment even after correction of hormonal imbalance should be evaluated to rule out organic causes of erectile dysfunction and be managed accordingly.

Conclusion

The abuse of AAS remains a major health concern all around the globe, with multiple organ system affection placing the abuser at risk for morbidity or even mortality. Efforts need to be employed not only to target individual black-market distributors, but also to prosecute and monitor their development and distribution by pharmaceutical companies. Moreover, raising the society's awareness, especially among adolescents, should help in alleviating this public health hazard. Fertility management of AAS/testosterone abusers is initiated with endocrine therapy to help stimulate testicular function and bridge subsequent testosterone deficiency. In the majority of patients, infertility secondary to AAS abuse is temporary with a documented improvement in semen parameters expected after a duration of up to 6 months from stopping the abuse.

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Chapter 5
Managing Infertility Due to Endocrine Causes

Philip Kumanov

Introduction

The testicular functions, the spermatogenesis, as well as the hormonal secretion are under the control of the hypothalamus–pituitary unit. Neurokinin B, kisspeptin, and dynorphin together are found in a subpopulation of neurons in the arcuate nucleus of the hypothalamus and are involved in the regulation of the gonadotropin-releasing hormone (GnRH) secretion [1]. Kisspeptin via its receptor GPR54 on the GnRH neurons in hypothalamus stimulates the release of GnRH [2]. The latter is secreted in a pulsatile fashion (1 pulse every 60–120–180 min) [1]. Only in such a manner of release is the stimulus effective. GnRH enters the pituitary portal circulation and reaches the gonadotroph cells in the anterior hypophysis, which release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Both are glycoproteins with the structure resembling those of thyrotropin-stimulating hormone (TSH) and of human chorionic gonadotropin (hCG). LH stimulates the testosterone biosynthesis in the Leydig cells, while FSH supports spermatogenesis and stimulates the secretion of both inhibin B and anti-Müllerian hormone (AMH) by the Sertoli cells [3]. Estrogens in men are mainly produced by peripheral conversion of androgens by the P450 aromatase. During and after puberty, testosterone via androgen receptors on Sertoli cells plays an important role in spermatogenesis and decreases the AMH production [3, 4]. The inverse relationship between testosterone and AMH persists from puberty into adulthood [4]. Sertoli cells via production of inhibin B decrease FSH secretion [5]. Testosterone directly as well as after aromatization to estradiol feeds back negatively on the LH secretion [1, 6]. This feedback on the LH involves not only the pituitary but partly also the GPR 54 and its ligand kisspeptin [2, 7]. The complicated machinery of the hypothalamus–pituitary–testicular axis is shown schematically in Fig. 5.1.

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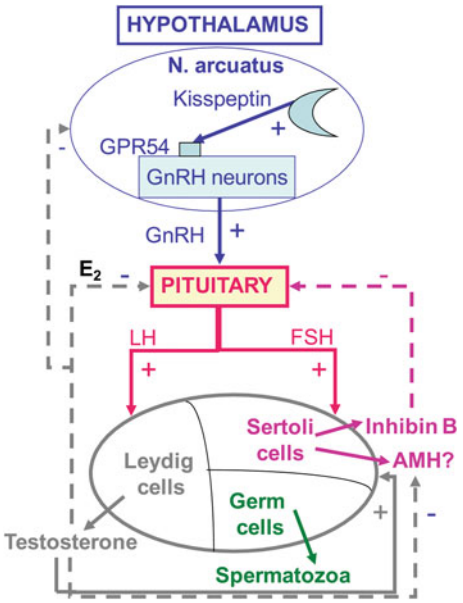

Fig. 5.1 The hypothalamo–pituitary–testicular axis. The negative influence is shown with *dash lines*. E_2 Estradiol; *FSH* Follicle-stimulating hormone; *LH* luteinizing hormone; *AMH* Anti-Müllerian hormone; "?" means that the action and site (s) of action are uncertain

Case Report

A 37-year-old man presents with severe oligoasthenoteratozoospermia (OAT): sperm concentration was 3 million/ml with 100% immotile sperms and 90% with abnormal morphology. His testes were evaluated by ultrasound: with normal volume and structure. There were no signs of varicocele. FSH and inhibin B levels were in the normal range: 2.24 IU/L and 187 pg/ml, respectively. After 3.5 months of treatment with clomiphene 25 mg/day (an estrogen receptor blocker) in combination with 1 capsule FertilAid (vitamins and antioxidants supplement) 3 times daily his spermiogramm revealed: sperm concentration 75 million/ml, 13% forward motility, and 65% with abnormal morphology.

This case illustrates that normal values of inhibin B and FSH were prognostic for the benefit of empiric therapy (this will be discussed in more detail in the Section "Therapy: Hormonal Treatment of Idiopathic Infertility").

Male Hypogonadism

Hypogonadism results from testicular failure (primary hypogonadism), or as a consequence of the hypothalamus and/or pituitary dysfunction (secondary hypogonadism). Male hypogonadism is a clinical syndrome of androgen deficiency and/or failure of producing normal number of spermatozoa with normal quality. Clinical

signs and symptoms of androgen deficiency include absent puberty, small testes, decreased or absent body and facial hair, decreased muscle strength, visceral obesity, decreased bone mineral density, depressed libido, reduced morning erections, erectile dysfunction, and male-factor infertility [8]. Hypogonadism can be further separated into congenital and acquired forms. Characteristics for the male hypogonadism are serum levels of total testosterone below the normal values of 12 nmol/l and/or free testosterone below 250 pmol/l [9]. Hypogonadism may not always be evident by low testosterone levels: some patients with primary testicular failure may have normal testosterone concentrations but high LH, which is referred as subclinical or compensated hypogonadism [8]. The prevalence of hypogonadism varies between 2.1 and 5.1% [1].

Among the most relevant forms of male hypogonadism of hypothalamic–pituitary origin are hyperprolactinaemia, isolated hypogonadotropic hypogonadism (IHH), and Kallmann syndrome (hypogonadotropic hypogonadism with anosmia) [8]. Male hypogonadotropic hypogonadism is rare with a prevalence 1 in 10,000 men [2]. The most widespread form of male hypogonadism of gonadal origin (primary hypogonadism) is Klinefelter syndrome with an incidence of 0.1–0.2% of male neonates [9]. Primordial germ cells degenerate early, so that by the beginning of the reproductive period of life the spermatogenesis is preserved only in very few seminiferous tubules.

Androgen receptor defects result in complete or partial androgen insensitivity syndrome [2] with infertility as an obligate feature.

The classification of hypogonadism is important also from therapeutic point of view: in males with hypogonadotropic (secondary) hypogonadism hormonal replacement therapy can successfully induce fertility [8].

Hyperprolactinemia

Prolactin is 23 kDA polypeptide hormone produced by the lactotrophs of the anterior pituitary; it is under tonic hypothalamic inhibition via dopamine D2 receptors [10]. Its physiological role in males remains unknown. A trophic effect of prolactin on male accessory glands is discussed [11]. While low prolactin may reflect a decreased serotoninergic signaling in the brain and is associated with a poor control of ejaculation [10, 11], elevated secretion of this hormone has serious pathological consequences. Processes causing pituitary stalk compression or section may give rise to hyperprolactinaemia because of the reduction or interruption of tonic negative dopaminergic influence of the hypothalamus on the pituitary lactotrophs [12]. High (>1500–2000 mU/L) blood levels of the hormone are often due to prolactin-secreting pituitary tumors (prolactinomas), which can be either microadenomas (less than 10 mm in diameter) and macroadenomas (more than 10 mm in diameter). Hyperprolactinemia can be caused also by pituitary tumors secreting both prolactin and growth hormone as well as by empty sella syndrome. Hyperprolactinaemia is observed in chronic renal failure as well as in primary

hypothyroidism where, because of the insufficient thyroid function sustained elevated thyrotropin-releasing hormone (TRH) secretion also may involve prolactin production. Hyperprolactinemia may also result from systemic use of drugs with dopamine antagonistic effects, such as metoclopramide and haloperidol, dopamine synthesis inhibitors (α[alpha]-methyldopa), opiates, calcium channel blockers, as well as medications stimulating prolactin synthesis and secretion (H2-blockers, estrogens). Whatever the cause, hyperprolactinaemia suppresses the gonadal axis on several mechanisms. It disturbs the pulsatile secretion of GnRH from the hypothalamus, which in turn decreases the FSH and LH secretion with testicular failure as a consequence [12]. Recently, it was shown that hyperprolactinemia via suppression of the kisspeptin decreases the GnRH secretion [13]. The tumor can also press and destroy the pituitary gonadotrophs. Direct effect of hyperprolactinaemia on the testes in men is still a matter of debate [2]. Symptoms of excessive prolactin secretion include depressed libido and erectile dysfunction; galactorrhoea in men and gynecomastia are rare. Usually, spermatogenesis is not heavily disturbed and often the sperm parameters remain in referent values. A study from Italy concluded that systematically evaluating prolactin levels in male infertility is not justified [11]. Because symptoms are usually not pronounced and not specific, hyperprolactinemia in men is usually discovered with some delay. This seems to be the cause for the higher prevalence of macroprolactinomas in men in comparison to the women. Hypoactive sexual desire as well as a hypogonadotropic hypogonadism raises the suspicion for hyperprolactinaemia.

Thyroid Diseases

Thyroid disorders are quite prevalent in people of reproductive age; 4–5 times more frequent in women than in men [14]. Thyroid hormone receptors have been described in human testis [14]. Since 1905 it has been observed that thyroid disorders, both hypo- and hyperthyroidism, affect the reproductive system [15].

Primary hypothyroidism (Table 5.1) results in a decrease of sex hormone-binding globulin (SHBG) and total testosterone concentrations, with free testosterone levels reduced in about 60% of hypothyroid males [15]. Elevation of serum prolactin concentrations might be observed. Normal FSH and LH levels and blunted gonadotropin responses to gonadotropin-releasing hormone indicate that in primary hypothyroidism the defect is not in the gonads but rather in the hypothalamus or pituitary [15]. Short-term post-pubertal hypothyroidism might decrease semen volume and sperm forward motility [14]. The conclusion of a prospective, controlled study was that in hypothyroidism only sperm morphology was significantly affected [15]. However, some years ago I treated a man with severe asthenozoospermia. He did not show any signs of hypothyroidism, but his TSH levels, measured routinely, were surprisingly high (>100 mIU/L). In the following months normalization of TSH levels was achieved with L-thyroxine replacement and sperm forward motility gradually increased without additional therapy.

Table 5.1 Parameters of male gonadal axis in thyroid disorders (hyperthyroidism and primary hypothyroidism)

Parameters	Hyperthyroidism	Hypothyroidism
Sex hormone-binding globulin (SHBG)	Increased	Low
Total testosterone	Increased	Decreased
Free testosterone	Reduced	Low
Estradiol	Increased	Normal
Luteinizing hormone (LH) basal levels	Increased or normal	Normal
Follicle-stimulating hormone (FSH) basal levels	Increased or normal	Normal
Gonadotropin responses to gonadotropin-releasing hormone (GnRH)	Exaggerated or normal	Blunted
Pulsatile secretion of LH and FSH	Co-pulsatility between LH and FSH more pronounced	

Thyroid hormone metabolism is mediated by cellular deiodinases, which belong to a large family of human proteins containing selenocysteine [16]. A multisystem selenoprotein deficiency disorder was described. Its features include male infertility, skeletal myopathy, hearing loss along raised free thyroxine, normal or low free triiodothyronine, and normal thyrotropin because of functional deiodinases deficiencies [16].

The interplay of *thyroid autoimmunity* and male infertility is not clear yet. Whether thyroid antibodies alter semen parameters directly still remains a matter of debate [14, 15].

In males with *hyperthyroidism* SHBG is elevated, total and bound testosterone are increased, free testosterone is reduced or without detectable changes, but the metabolic clearance rate of testosterone is reduced; circulating estradiol levels are elevated (Table 5.1) [14, 15, 17]. The lack of unbound sex steroids in hyperthyroid conditions is more pronounced in male patients than in women, probably because of the higher affinity of SHBG for testosterone than for estradiol [17]. The response of Leydig cells to hCG administration is blunted [15]. Disturbances in the gonadal steroid equilibrium with the estrogen increase and decreased free testosterone/free estradiol ratio [17] might be the cause of gynecomastia, which can develop in the course of thyreotoxicosis. In an extensive study of men with Grave's disease [17] more pronounced co-pulsatility of LH and FSH was observed than in the controls (Table 5.1). In the same study, results of GnRH stimulation testing were preserved showing adequate gonadotropin reserve capacity of the pituitary, which suggests intact function of the hypothalamic–pituitary unit and even more coordinate secretion of hypophyseal hormones [17]. The gonadal insufficiency is due to the increased need for sex steroids to compensate for elevated SHBG levels, thus presenting a relative endocrine testicular insufficiency, which may explain the infertility in longstanding hyperthyroidism [17]. Asthenozoospermia can be revealed in men with untreated Grave's disease [17]. In a prospective study of 23 hyperthyroid males the semen volume was unchanged, sperm density, sperm

motility and morphology were lower in comparison to the healthy men; after treatment of thyrotoxicosis motility normalized but not morphology [18].

Patients with thyroid abnormalities report decreased libido, erectile dysfunction, and ejaculatio praecox or delayed ejaculation [14, 15, 17].

Radioactive iodine (I^{131}) is widely employed in the treatment of hyperthyroidism and thyroid cancer [14]. The radiation leads to transient suppression of spermatogenesis along with temporarily elevations of FSH serum levels and inhibin B suppression [14, 15].

All these data suggests that screening for thyroid abnormalities in males with disorders of spermatogenesis and/or hypoactive sexual desire and erectile dysfunction is justified.

Obesity and Metabolic Syndrome

Both abnormally low and high body mass index (BMI) are associated with disturbances in spermatogenesis.

Obesity has increased worldwide over the last decades. Overweight and obese couples are at a higher risk of being infertile [19]. The metabolic syndrome (MTS) represents the clustering of abdominal (visceral) obesity, insulin resistance, dyslipidemia, and elevated blood pressure and is associated with other comorbidities among which are reproductive disorders [20]. MTS is increasing to a worldwide epidemic affecting developed as well as developing countries. Men with MTS appear to have a greater prevalence of hypogonadism; on the other hand androgen insufficiency seems to be a risk factor for the development of MTS and type 2 diabetes mellitus [20]. In a large meta-analysis it was found that men with low concentrations of total testosterone, SHBG, and free testosterone were more likely to have MTS compared to those having high sex hormone levels [21]. It is not clear which components of MTS cause the hypogonadism. As hypogonadotropic hypogonadism is rare in patients with diabetes mellitus type 1, impairment of the gonadal axis in those with diabetes mellitus type 2 probably is not mediated only by hyperglycemia [22]. Large prevalence of hypogonadotropic hypogonadism was recently proved in males with moderate to severe obesity [23]. According to Brand et al. [21], total testosterone was most strongly associated with hypertriglyceridemia and abdominal obesity. Total testosterone, SHBG, and free testosterone fraction in obese men are lower in comparison to lean men. Peripheral conversion of testosterone to estrogen in increased peripheral adipose tissue may lead to elevated circulating estrogen levels, which via negative feedback decrease the gonadotropin secretion thus causing hypogonadotropic hypogonadism [24, 25]. Excessive adipose tissues have elevated aromatase activity and adipokines production. The relationship between adipose tissue and estradiol might be modulated by the aromatase polymorphism [19]. In obesity there is an increase of leptin, the obese gene product secreted from adipocytes. Its receptors are present in testicular tissues [25]; therefore, excessive leptin may be another factor for reducing

androgens in obese men. The kisspeptin system being superior to GnRH neurons in the hierarchy of the neuroendocrine regulation triggers the GnRH secretion. Functional leptin receptors are not expressed in GnRH neurons, but kisspeptin neurons have leptin receptors [22], and this may be the site of central inhibiting action of excessive obesity. There is inverse relationship between serum total testosterone, free testosterone, and SHBG with visceral fat [25]. Viscerally accumulated fat can serve as a major endocrine disrupter [19]. It is associated with elevated concentrations of insulin and glucose. Increased insulin reduces the SHBG levels. Our study has found that in young males with MTS total testosterone was significantly lower compared with nonobese age-matched subjects and negatively correlated with insulin level, insulin resistance, and BMI [26]. Insulin resistance and maybe also hyperglycemia appear to influence negatively the GnRH pulse generator [22]. Hypotestosteronemia in obesity and MTS seems to be due to several factors, among them the decreased testosterone production, the suppressed gonadotropin secretion, and inhibition of SHBG synthesis [24]. The cause-and-effect relationship between obesity, insulin resistance, and type 2 diabetes mellitus on one side and androgen deficiency on the other remains unclear. Stronger associations of sex hormones with prevalent than incident MTS found in the already mentioned large meta-analysis by Brand et al. suggest that low testosterone and SHBG are merely a result rather than cause of MTS [21]. Weight loss has been associated with an increase in testosterone and SHBG levels in obese men with MTS. Polymorphisms in the SHBG gene have been associated with risk of type 2 diabetes mellitus [21], which puts forward the possibility that this career protein may be a causative factor for MTS. In addition a great body of evidence has accumulated in the last years that testosterone replacement therapy in hypogonadal men reduces visceral fat and increases lean body mass, as well as is associated with reduction of blood glucose. All these data suggest the bidirectional relationships between reproductive system and MTS [21].

Obesity as well as MTS is associated with disturbances in spermatogenesis: decreased sperm concentration and motility and increased sperm DNA damage [24]. Moreover, we have found decreased levels of anti-Müllerian hormone and inhibin B in patients with MTS, which suggests impairment of the Sertoli cells function [27]. They can be a result of altered testosterone secretion and elevated estrogen levels due to overweight and obesity, but alterations in reproductive hormones might not fully explain the poor semen quality in obesity [19]. Leptin receptors are present on the sperm membrane [19], so in obesity and MTS increased leptin may contribute to infertility. Another important factor for sperm disturbances could be the oxidative stress due to the inflammatory adipokines and dyslipidemia [19, 24]. Toxins from the closest environment (excessive scrotal skin) may have a pathogenic role for the spermatogenesis [19]. Suprapubic and thigh fat are factors for the increase of scrotal temperature in severely obese men [24].

Evaluation of an Infertile Man from an Endocrinological Point of View

In any infertility couple, regardless of the suspected cause, the male factor should be evaluated simultaneous to the female partner. Similar to any other medical condition, work-up starts with a detailed history. Attention should be paid to cryptorchidism and/or testicular torsion, pubertal development, scrotal trauma, and/or radiation or surgeries in this region, as well as past or current use of tobacco, anabolic steroids, and alcohol consumption [28]. Physical examination includes body height and weight, body proportions, hair distribution, testicular size (evaluated with orchidometer or by ultrasound), and presence of gynecomastia or varicocele.

When the patient is able to ejaculate, semen quality should be determined in accordance with World Health Organization (WHO) guidelines and standards [29].

An endocrine evaluation is mostly indicated for men with sperm density below 10 million/ml, impaired sexual function, and other clinical findings indicating specific hormonal disorder [28]. Because of the circadian variations in secretion, serum samples for hormone levels should be obtained in the morning between 8 a.m. and 11 a.m. In healthy men of reproductive age, serum levels of testosterone are above 12 nmol/l. Hypogonadism is marked by testosterone serum levels below 8 nmol/l, while the values between 8 and 12 nmol/l require further evaluation [2]. Liquid chromatography—tandem mass spectrometry—is the strongly recommended method for testosterone determination but the conventional methods are still in use in the routine practice [1, 2]. About 2% of total testosterone in circulation is not bound. This is the free, biologically active fraction of testosterone. For estimation of the free testosterone the gold standard is the equilibrium dialysis [25], which is a complicated method not routinely used at present. Morning salivary testosterone correlates well with free testosterone [25]. Free testosterone level can also be derived from measuring total testosterone and SHBG [30]. This is particularly important when alterations in SHBG are expected and thus making the diagnostic values of the serum total testosterone concentration questionable as in obese patients or those with nephrotic syndrome, hyperthyroidism or hypothyroidism, chronic liver disease, on therapy with steroids, or in older men [1, 31].

Low testosterone and elevated FSH and LH serum levels suggest a testicular origin of male hypogonadism (primary testicular failure), whereas patients with intact Leydig cell function and isolated spermatogenic failure have normal testosterone and LH, but elevated FSH values. In men with FSH levels in the upper normal range, there may be impaired spermatogenesis as well [28]. Low testosterone and low or inappropriately normal LH and FSH (hypogonadotropic hypogonadism) are typical for hypothalamic or/and pituitary causes of hypogonadism (secondary or hypogonadotropic hypogonadism). In these situations prolactin, TSH, and free thyroxine levels should be measured. Brain imaging is always indicated in hypogonadotropic hypogonadism to exclude or reveal a process in the hypothalamic–pituitary region.

Normal prolactin levels in men are usually less than 500 mIU/L. However, due to high assay variability, testing should be repeated if levels are elevated. However, false-positive results (10–22% of all hyperprolactinaemia) are due to high molecular forms (macroprolactinaemia), a biologically inactive complex of prolactin with immunoglobulin G [2, 10]. Screening for macroprolactinaemia either by chromatography or by precipitation with polyethylene glycol is indicated in cases with negative history of drug consumption, normal testosterone levels, and intact sexual function [10].

The gonadotropin-releasing hormone (GnRH) is a useful method for evaluation of the pituitary gonadotropin reserves. It is indicated when basal LH and FSH levels are close to lower normal range. In healthy men LH increases to 3–5 times the basal concentration at 15–30 min after intravenous application of 100 μ(mu)g GnRH [2]. Failure of SHBG to decrease after testosterone administration confirms the androgen insensitivity. The test with hCG can be used to evaluate the Leydig cell capacity of androgen production. In men who are found to have azoospermia but normal testosterone, LH, and FSH levels and normal testes volume, obstructive disorders should be ruled out by measuring seminal levels of neutral alfa-glucosidase. The latter originates in the epididymis [29] and its low seminal levels in men with normal testicular volume and normal FSH are indicative for obstructive azoospermia (bilateral obstruction of the epididymis and/or vas deferens or congenital aplasia of the vasa deferentia). In these situations genetic screening for cystic fibrosis gene mutations is justified.

Inhibin B is a heterodimeric glycoprotein produced by the Sertoli cells [32]. Its serum levels have been found to correlate better with sperm parameters than FSH and thus may serve as a better marker of spermatogenesis [33]. It is very useful for the prognosis in cases with FSH levels in the upper normal range [33]. Inhibin B levels can also be useful for monitoring the effects of gonadotropin therapy.

Recently, growing attention is granted to the anti-Müllerian hormone (AMH), which is produced by Sertoli cells [34], and therefore reflects their status and interplay with other testicular compartments. Its specific role during puberty and reproductive years for AMH in males remains to be established. The AMH serum levels are extremely high in congenital and untreated hypogonadotropic hypogonadism due to low testosterone secretion. AMH is also elevated in cases with cryptorchidism [2]. Today, it is still not clear whether AMH might be a useful additional contributor to more precise diagnosis and prognosis of male infertility. However, in men with a history or presence of maldescended testes AMH can serve as a marker of Sertoli cell number and function [34]. A recent prospective study proposed that seminal AMH can help to select those patients who may benefit from therapy with recombinant FSH [35].

In cases with azoospermia or severe oligozoospermia, normal testosterone, and LH levels, but elevated FSH, primary spermatogenic failure should be considered. These patients require testicular ultrasound assessment, karyotyping, and screening for Y-chromosome microdeletions.

Androgen receptor studies are reserved for cases suspected for testosterone insensitivity.

Therapy

While the lifestyle intervention with overweight loss is of obvious benefit for the general health, including the increase of testosterone levels, the effect of weight normalization for the improvement of spermatogenesis is not clear. Recently, it was shown that correction of hypogonadism after metabolic surgery is accompanied by a decrease of glucose levels as well as reduction of insulin resistance [23]. For the successive treatment of infertility in patients with metabolic syndrome and type 2 diabetes, the obligate prerequisites are normalization of the weight and stable blood glucose levels within the reference range. Only then the specific therapeutic approach can be introduced.

Treatment of Male Hypogonadism

Exogenous testosterone reduces the testosterone production in the gonads by the negative feedback on the hypothalamic–pituitary unit. Therefore, in cases with hypogonadotropic hypogonadism when the fertility is aimed, testosterone treatment is not indicated. Instead human chorionic gonadotropin (hCG) should be introduced; it corresponds to LH activity [2]. The recommended doses vary between 1500 and 5000 IU administrated intramuscularly (im) or subcutaneously (sc) twice weekly [8]. In patients with secondary (hypogonadotropic) hypogonadism hCG should be combined with FSH treatment 150 IU 3 times weekly or 225 IU twice weekly im or sc to induce spermatogenesis [2, 8, 36]. It can be used with either recombinant FSH (Gonal F, Puregon) or human menopausal gonadotropin (hMG). Recombinant and urinary FSH seem to be equally effective and safe, but the former does not lead to formation of detectable antibodies [37]. The patients are treated to the sperm appearance in the ejaculate or to the occurrence of pregnancy [2]. Due to the physiologically long-lasting human spermatogenesis the treatment in men should continue 6–9 and more months, 2 or more years. Once a treatment cycle has been completed, time to re-induction of fertility is reduced to 6–10 months when repeating this treatment later [38]. History of cryptorchidism or presence of small testes is not a reason not to induce such therapy [2], but they seem to be negative predictors of therapeutic outcomes [38]. In a retrospective analysis on patients with different forms of hypogonadotropic hypogonadism, treated with gonadotropins between 1998 and 2015, it was shown that those with an early impairment of the gonadal axis during fetal life had the poorest therapeutic outcomes, whereas men with unaffected development until early puberty achieved better results, so that the underlying cause of hypogonadotropic hypogonadism is a predictor of outcome of gonadotropin replacement in adults [38]. Surprisingly, there are many reports for successive pregnancies with low sperm concentration under this therapeutic stimulation [2, 38].

A meta-analysis from Italy confirmed that gonadotropin therapy, even with urinary products, was able to induce and restore spermatogenesis in hypogonadotropic subjects and showed that previous use of testosterone as replacement did not affect the results obtained with gonadotropin therapy [39].

When the pituitary is intact and the hypogonadotropic hypogonadism is caused by GnRH deficiency, the spermatogenesis can be stimulated also with GnRH. It can be effective only if applied in a pulsatile manner using a portable minipump with a fine needle inserted subcutaneously on the abdomen. A starting dose of 4 μ(mu)g per pulse should be increased if there is not a rise of LH to the maximum dose of 20 μ(mu)g per pulse [40]. The duration is the same as with the gonadotropin therapy [41]. In such patients this therapy most closely resembles physiological regulation. However, this treatment does not show significant superiority to the gonadotropin replacement therapy. Both gonadotropin and GnRH therapy are effective for the induction of spermatogenesis in hypogonadotropic hypogonadal men [41]. GnRH is ineffective in patients with pituitary insufficiency and also in rare cases of GnRH receptor mutations that cause resistance to GnRH [40].

Fertility can be restored in hypogonadotropic hypogonadal patients with gonadotropin or with GnRH therapy and the pregnancy rates vary between 50 and 90% [40]. Once the spermatogenesis is initiated in men with hypogonadotropic hypogonadism, it can be maintained with hCG alone [42].

Not all patients are able or willing to wear the infusion minipump a long time as it is required [40]. On the contrary, patients learn to self-administer gonadotropin preparation subcutaneously. This treatment is preferable over the GnRH therapy because of an easier and technically not so complicated route of administration. Therefore, today the gonadotropin replacement is the first-line therapy for inducing spermatogenesis in patients with hypogonadotropic hypogonadism [43]. It is a well-tolerated and effective mode of treatment.

Treatment for infertility of patients with Klinefelter syndrome has improved dramatically in the last years. Some seminiferous tubules may have preserved sperm that can be obtained by biopsy and introduced directly into the ova; however, the risk of aneuploidy in these offsprings is increased [9].

Hormonal Treatment of Idiopathic Infertility

Recombinant FSH alone is used in normogonadotropic subjects with isolated idiopathic oligozoospermia [44, 45]. In one study, positive results were achieved with high doses of recombinant human FSH: 300 IU every other day for a period of 4 months or longer [46].

At present the core of hormonal empiric treatment is the alteration of estrogen production or action. The aim is to weaken the estrogen negative feedback on the hypothalamic–pituitary unit. As a result Leydig cells increase the secretion of testosterone, while FSH via Sertoli cells augments the spermatozoa production.

Estrogen receptor blockers (antiestrogens, estrogen antagonists) clomiphene citrate and tamoxifen are widely used as empiric treatment for male idiopathic infertility [47]. Additional argument for their use is that they may counteract the adverse effects of environmental xenoestrogens [43]. However, the reports on the role of estrogen antagonists in males are controversial. The daily dose of clomiphene citrate is 25 or 50 mg, and that of tamoxifen is usually 20–30 mg. The positive effect on sperm count seems to be modest. Reported adverse events are rare and include headache, skin rush, dizziness, and visual disturbances. A meta-analysis of randomized controlled trials concluded that these medications may improve sperm concentration and motility and thus the spontaneous pregnancy rate [47].

Estrogen antagonists are also used in different combinations for treatment of idiopathic infertility. Encouraging results on sperm concentration and motility were noted with clomiphene citrate (25 mg daily) and vitamin E (400 mg/day) as an antioxidant for 6 months in men with idiopathic oligoasthenozoospermia [48]. A large study demonstrated beneficial effects on sperm parameters and spontaneous pregnancy rate by combining tamoxifen with testosterone undecanoate for 6 months [49].

It was attempted to improve the male fertility by influencing the estrogens but in quite a different way. Aromatase inhibitors prevent the conversion of testosterone into estradiol [50]. There are two types of aromatase inhibitors: steroidal (testolactone) and nonsteroidal (anastrozole and letrozole) [43]. A placebo controlled study showed no improvement in semen quality when testolactone was used; however, in a single case with azoospermia spermatogenesis was induced with 2.5 mg letrozole orally daily for 4 months [50]. Aromatase inhibitors are suitable for infertile men with extreme obesity because in these cases absolute or relative hyperestrogenemia is present but this treatment is associated with increased risk of developing osteoporosis [43].

Treatment of Hyperprolactinemia and Thyroid Disorders

When possible, use of the drug suspected to cause hyperprolactinemia should be stopped. Oral dopamine agonists are the first-line medication for prolactinomas [51] as well as for nontumorous hyperprolactinemia. Historically, bromocriptine (2.5–10.0 mg daily) was the fist effective medical therapy. Cabergoline in dosage of 0.25–3.0 mg weekly is better tolerated than bromocriptine [51]. Both normalize prolactin levels, shrink the prolactinomas, and restore reproductive function. However, there are some concerns of heart valvular abnormalities with higher doses of these medications. Because of its high affinity for serotonin receptor 2B (HTR2B) located on the heart valves, cabergoline may lead through activation of these receptors to fibromyoblast proliferation [51, 52]. Individual susceptibility may play a role since there are polymorphisms of the serotonin receptor [52].

Trans-sphenoidal surgery is indicated when the tumor does not respond to the dopamine agonists, in cases of intensive growth of the adenoma with compression of the optic chiasm, as well as in pituitary apoplexy [12]. Radiation therapy is occasionally used in cases when surgery and dopamine agonist treatment fail to restore normal prolactin secretion [51].

The abnormalities in the hypothalamic–pituitary–testicular axis both in hyperthyroidism and hypothyroidism revert with the normalization of thyroid hormone levels and restoration of the euthyroid status, so no specific treatment for the reproductive disorders is required [15, 53].

Conclusion

With a better understanding of the fine mechanisms of hormonal interrelationships and with introducing modern diagnostic methods, the uncertain empiric approach will undoubtedly retreat to make room for the successful treatment of male infertility.

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Chapter 6
Management of Infections in Male Infertility

Odunayo Kalejaiye and Asif Muneer

Introduction

Male factors are estimated to be responsible for up to 50% of couple infertility, with 8–35% of these being due to an underlying genital tract infection [1]. Genital tract infections may present acutely, be part of a chronic disease, or manifest subclinically. Organisms may cause infertility by:

- suppression of the hypothalamic–pituitary–gonadal axis
- impairment of spermatogenesis
- obstruction of the genital tract
- inhibition of accessory gland function
- raising the inflammatory response resulting in free radical injury to the sperm

In addition, generalized febrile illness may also be implicated in causing temporary subfertility. The diagnosis of infection requires a thorough history, clinical examination, semen analysis and semen culture, urethral swabs, or urine culture.

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Sites of Infection

Urethritis

Urethritis is most commonly due to sexually transmitted infections (STIs) and may be divided into 2 main groups:

- Gonococcal (*Neisseria gonorrheae*)
- Non-gonococcal (*C. trachomatis, Mycoplasma, Trichomonas vaginalis*) [2, 3]

Chlamydia is a major cause of urethritis, being responsible for an estimated 30% of all cases of urethritis [4]. Urethritis may present with dysuria, urethral discharge, erythema of the glans or external urethral meatus, or it may remain asymptomatic [2–4]. The diagnosis is based on gram staining of a urethral smear (>4 leukocytes per microscopic field ×1000) and the presence of leukocytes in the first part of a voided urine specimen (>15 leukocytes per microscopic field ×400) [2, 3]. Once diagnosed the treatment requires a single dose of ceftriaxone and either a 1-week course of doxycycline or a single dose of azithromycin [2, 3]. All men with suspected STI should be advised to attend an appropriate clinic for both contact tracing and screening for other STIs [5]. Urethritis has not been conclusively proven to be associated with male infertility [5].

Epididymitis

Epididymitis is one of the commonest causes of an acute scrotum with an incidence of 25–65 cases per 10,000 adult males per year [2, 5]. It may present acutely, chronically, or recurrently, with some men having had an exposure several months prior to the development of symptoms [2, 3, 5]. Chronic epididymitis may occur in up to 15% of men [3]. The responsible organisms depend on the patient's age, previous urinary tract instrumentation, or anatomical abnormalities, which may increase their risk of a urinary tract infection [2, 3]. Men who are younger than 35 years are more likely to be infected with STIs, with chlamydia and gonorrhea being the most frequent [2, 3, 5, 6]. Chlamydia is 2–3 times more common than gonorrhea [2]. However, older men are more likely to be infected by the common urinary tract organisms such as *Escherichia coli* (*E. coli*) or *Pseudomonas* [2, 3, 6]. Other organisms that may cause orchitis include mumps virus if there are prodromal symptoms and tuberculosis (TB) in men with immunodeficiency or if they are from high-prevalence countries [5]. These organisms can travel retrogradely from the urethra and into the ejaculatory ducts and vas to eventually infect the epididymis or testicles [6].

Epididymitis may present with systemic features in 30–50% of cases, scrotal pain, and scrotal swelling [2, 3]. The diagnosis is obvious on ultrasonography with the classic features of an enlarged and hypoechoic epididymis and hypervascular

Table 6.1 Treatment of epididymitis or orchitis

Low risk of gonorrhea	Flouroqinolone active against chlamydia once daily for 10–14 days OR Doxycycline AND antibiotic against enterobacteriaceae for 10–14 days
Likely gonorrhea	Single dose ceftriaxone intramuscularly AND doxycycline for 10–14 days
Non-sexually transmitted infection	Fluroquinolone for 10–14 days

testicular parenchyma [3]. Semen or urine cultures or urethral swab may identify the causative organism [5]. The treatment will depend on the underlying organism (see Table 6.1) [5].

Acute epididymitis may be associated with a transient reduction in the sperm concentration and forward motility, which may normalize following a course of treatment with antimicrobials [2, 5]. Following epididymitis, the resultant scarring can cause epididymal obstruction, which is the most frequent cause of obstructive azoospermia affecting 30–67% with azoospermia [3, 5, 6].

Orchitis

This is another common cause of the acute scrotum, which usually coexists with epididymitis. The causative agents may be subdivided into viral and bacterial [2].

Viral orchitis usually arises from hematogenous spread and may be caused by paramyxovirus (the virus that results in mumps), coxsackie, varicella, lymphocytic choriomeningitis, and Marburg virus [2, 3].

Mumps orchitis is rare in prepubertal boys but may occur in 15–30% of pubertal or post-pubertal men [2, 3, 7]. Both testes are involved in approximately a third of all cases and orchitis follows a period of parotitis in most cases [2, 3]. Mumps orchitis may be associated with infertility in 25% of bilateral cases and testicular atrophy is a sequelae in 30–50% of affected testicles [2, 7]. The virus may be isolated from saliva, urine, blood, nasopharyngeal swabs, and seminal fluid within 1 week of the onset of symptoms [7]. The treatment is mainly supportive with scrotal elevation and cooling, anti-inflammatory, and antipyretic agents [3, 7].

Bacterial orchitis may be caused by spread of bacteria from the epididymis [2]. Common pathogens include gonorrhea, chlamydia, gram-negative bacilli (*E. coli*, *Klebsiella pneumoniae* [*K. pneumoniae*], *Pseudomonas*) and gram-positive cocci [2]. Organisms such as syphilis, tuberculosis, and leprosy should also be considered in populations from high-risk countries as they are now unusual in industrialized countries [2]. The treatment is the same as for epididymitis [2, 3].

Orchitis may present with testicular swelling, pain, fever, and an acute hydrocele [2]. Acute infections may be associated with a transient reduction in semen

parameters, which often recover after resolution of the infection [3]. In addition orchitis may affect fertility by the direct effect of the organism as well as a pressure-induced necrosis of the seminiferous tubules due to parenchymal edema within the testicle [3, 4, 6, 7].

Prostatitis

Prostatitis is classified according to the National Institutes of Health (NIH) into types I–IV (see Table 6.2) [2, 3, 5]. Common pathogens include: *E. coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Enterococcus* sp.; *E. coli* is the most common organism isolated in bacterial prostatitis [2, 3].

Acute prostatitis may present with fever, dysuria, and rectal and perineal pain [3]. The diagnosis may be suspected in men with positive urine cultures or the development of a prostatic abscess on transrectal ultrasonography (TRUS) [3]. A leukocyte count of greater than 10–15 per high powered field (HPF) ($\times 1000$) in expressed prostatic secretion (EPS) or ≥ 3 leukocytes per HPF in urine after prostatic massage is considered a significant finding [3]. Chronic prostatitis is more difficult to diagnose but may be suspected if the number of organisms in the urine is 10 times higher after prostatic massage compared with the count prior [3].

The treatment of prostatitis is usually with extended periods of antibiotics; up to 6–12 months in some men [2]. Acute prostatitis should be treated with at least 4 weeks of broad spectrum penicillin, third-generation cephalosporins, or fluoroquinolones with aminoglycosidase [3]. Chronic prostatitis may be treated with at least 4–6 weeks of fluoroquinolones; this may need to be extended if the patient remains symptomatic [3].

Inflammation of the seminal vesicles (vesiculitis) often occurs together with prostatitis (prostatovesiculitis) [3]. The symptoms are similar to prostatitis but may be suspected if the seminal gland and prostate have a pasty consistency on digital rectal examination [3].

Table 6.2 National Institutes of Health (NIH) classification of prostatitis

Type I	Acute bacterial • Leukocytes and uropathogens in MSU
Type II	Chronic bacterial • Leukocytes and uro-organisms in EPS or urine after prostate massage • Chronic/recurrent infections
Type IIIA	Chronic abacterial • Leukocytes in EPS, urine after prostate massage or semen
Type IIIB	No leukocytes or organisms
Type IV	Asymptomatic inflammatory • Leukocytes in EPS, urine after prostate massage or in semen

MSU Midstream urine
EPS Expressed prostatic secretion

Prostatitis and prostatovesiculitis may both result in obstruction of the ejaculatory ducts, increased reactive oxygen species (ROS), and increased sperm DNA fragmentation [3, 6, 8, 9]. A semen sample with a low volume, azoospermia, negative fructose, and acidic pH is diagnostic for ejaculatory duct obstruction (EDO) [3, 5, 6, 9]. Chronic bacterial prostatitis (CBP) is associated with a significant reduction in sperm vitality and motility [8, 9]. It has been suggested that spermatozoa damage is mediated via the influx of inflammatory markers such as leukocytes, cytokines, oxidative stress, and reactive oxygen species [8, 9]. Antibiotic treatment may have a temporary negative effect on spermatogenesis and sperm function [3].

Types of Organisms

Neisseria Gonorrhea

Neisseria gonorrhea is a gram-negative intracellular diplococcus, which is a common cause of urethritis [2]. Most infected men will be symptomatic, with only 10% of those infected being asymptomatic [2]. Ascending infection of the genital urinary tract is rare with the exception of the urethra. However, infection of the epididymis or testis may result in testis damage, epididymal obstruction, and significantly raised seminal leukocytes [2].

Chlamydia trachomatis

Chlamydia trachomatis (Ct) is the most common STI in industrialized countries with an estimated incidence of 92 million worldwide in 1999 [2, 10]. A systematic review in the UK reported a prevalence of 11% in men [11]. It is estimated that up to 50% of men infected may be asymptomatic, with only 5% of those diagnosed treated compared to 25% in women [2, 12, 13]. The result of these figures is that the infection is allowed to persist in the general population, enabling further spread and disease [3, 4]. In addition a recent study has suggested that early diagnosis and treatment may be associated with higher reinfection rates; the authors further add that the only viable option for long-term eradication is the development of a vaccine [14].

C. trachomatis (Ct) is associated with significant genital pathology and may be a cause of urethritis, epididymitis, orchitis, and prostatitis [2, 4]. There remains controversy as to whether Ct causes prostatitis [2, 4]. Semen may be contaminated by passage through the urethra infected with Ct [4]. However, several studies have now detected Ct in up to 30% of prostate biopsies where there was no urethral infection [15–18].

The role of Ct in infertility is also controversial. *C. trachomatis* attaches to sperm in vitro resulting in an increase in ROS and caspase-mediated apoptosis [4]. This interaction may result in a reduction in sperm motility and sperm density [2, 4]. This has been supported by a large number of studies, which have revealed that Ct is associated with a reduction in:

- normal sperm morphology,
- semen volume,
- sperm density, and
- motility [2, 19–26].

C. trachomatis has also been shown to be associated with an increase in DNA fragmentation, the production of anti-sperm antibodies (ASA), reduction in α(alpha)-glucosidase, and an increase in leukocytes [2, 3, 26, 27]. α(alpha)-glucosidase is a marker of epididymal function. In addition, coinfection with *Mycoplasma* resulted in a 3.2-fold greater incidence of DNA fragmentation in a study by Gallegos et al. [28]. A different study of men with at least 6 months of chronic prostatitis-related symptoms and coinfection with human papillomavirus (HPV) resulted in a significant reduction in motility, normal morphology, and an increase in proportion with subfertility [27]. Other studies have found none of the alterations described [4, 29–35]. However, this may have been due to diagnostic difficulties with Ct [2].

Mycoplasma

Genital ureaplasma (*U. urealyticulum* and *U. parvum*) and genital mycoplasma (*M. genitalalium* and *M. hominis*) are natural inhabitants of the male urethra [36]. They may contaminate semen during ejaculation. However, only *U. urealyticulum* (UU) and *M. hominis* are pathogenic in humans [2, 36].

U. urealyticulum is a significant cause of urethritis. Its presence in semen was first described by Gnarpe and Friberg when they demonstrated a higher prevalence in the semen of infertile men (76%) compared with fertile men (19%) [37, 38]. *U. urealyticulum* attaches to the head and mid-piece of spermatozoa, reducing motility and increasing ROS and DNA damage [2, 39]. Overall, studies have also demonstrated that UU is associated with semen with increased viscosity and lower pH [39]. Treatment of UU has, in some studies, resulted in improved motility and pregnancy rates; however, this finding has not been uniform [40–42].

Herpes Virus Family

The herpes group of viruses consist of:

- Herpes Simplex virus (HSV)
- Cytomegalovirus (CMV)
- Epstein–Barr virus (EBV)
- Varicella zoster virus (VZV)
- Human herpes virus (HHV) [2]

In a study by Bezold et al. [26], which reviewed the prevalence of STIs in asymptomatic infertile males, herpes simplex viruses were the most frequent with CMV most common. All the STIs studied were associated with a significant reduction in motility and total sperm count; there was a nonsignificant trend toward a reduction in sperm density [2, 26, 43]. HSV was the only STI associated with a significant reduction in density, citrate, and α(alpha)-glucosidase [2, 26, 43]. Citrate is a marker of prostate function. This suggests that HSV may also have an adverse effect on prostate and epididymal function [26]. HSV is also implicated in raised levels of ROS and leukocytospermia [2, 43].

Human Papillomavirus

HPV has a well-established role in the development of genital disease in both sexes [2]. In a study of 104 men attending an infertility clinic, 41% had seminal HPV [44]. In another study of 185 men with azoospermia undergoing testis biopsy, 6.5% had HPV isolated compared with 0% of normal controls [45]. However, each of the patients had other possible causes for their infertility [45]. This would suggest a possible role for HPV in the etiology of infertility. A systematic review, including 9 studies evaluating the effects of HPV on semen analysis, demonstrated a reduction in motility (8 studies) and sperm count (9 studies) [2, 46, 47]. The effects on morphology are a more mixed picture [2, 46, 47].

Human Immunodeficiency Virus

Human immunodeficiency virus (HIV) is a cause of significant morbidity worldwide. Early infection is not usually associated with semen abnormalities; however, progression to acquired immunodeficiency syndrome (AIDS) is associated with impaired sperm density, morphology, motility, and semen viscosity [2]. There is also an increased level of leukocytospermia [2, 43]. HIV is also associated with symptomatic androgen deficiency [2]. The prevalence increases with progression to AIDS and with the clinical status of the disease [3]. The advent of highly active

antiretrovirals (HAART) has reduced the prevalence dramatically [3]. However, it is unclear if these effects on fertility are due to the virus or to the use of antiretrovirals [2, 3].

Mumps

Mumps is a contagious RNA virus belonging to the Paramyxoviridae family [7]. Prior to the introduction of a vaccine in the 1960s, it commonly affected children between the ages of 5–7 years with a global prevalence of 290 cases per year per 100,000 population between 1977 and 1985 [7]. Following the introduction of the vaccine, the incidence fell by a reported 99% in the USA [7]. Unfortunately, there has been a recent resurgence in the virus, which started in 2004 due to sustained negative media coverage about the vaccine [7]. This resulted in a reduction in vaccine uptake from 91 to 55% in some urban areas of the UK. This resurgence in the virus has mainly affected adolescents and young adults aged 15–25 years [7].

The effect on male fertility is not completely clear. There is direct infection of the seminiferous tubules with resultant infiltration of inflammatory markers and necrosis of the tubules [3, 7]. This may lead to fibrosis and testicular atrophy [3, 7]. Direct testicular damage may also result in a reduction in serum testosterone and an increase in gonadotrophins [3, 7]. However, there is conflicting evidence about the effect on testosterone and gonadotrophins [7]. Abnormal spermatogenesis has been reported in up to 50% for up to 3 months post-recovery [7]. Abnormal sperm count, motility, and morphology have also been reported [7].

Subfertility is reported in 13% and may occur without testis atrophy [7]. Infertility is rare [7].

Other Organisms

See Table 6.3 for other organisms that cause infections [2, 3].

Table 6.3 Other organisms [2, 3]

Trichomonas vaginalis	• Sexually transmitted infection • Causes a minority of non-gonococcal urethritis • Reduced motility and morphology • Treatment: metronidazole
Lepromatous leprosy	• Testis atrophy • Hypogonadotrophic hypogonadism
Tuberculosis	• Epididymitis • Hypogonadotrophic hypogonadism Treatment: triple therapy

Markers of Inflammation

Leukocytospermia

The testes are immunologically sequestered organs [2]. This is achieved through Sertoli cells' tight linkage, which creates a blood–testis barrier [2]. Several authors have described the constituents of the normal genitourinary tract. The epithelial lining of the prostate, seminal vesicles, and epididymis contain mainly lymphocytes; however, the testes do not contain any lymphocytes [2]. An abnormal concentration of leukocytes ($>1 \times 10^6$ WBCs/mL of semen) in the semen (leukocytospermia) may indicate a pathological process contributing to infertility (Table 6.4) [2]. The most common leukocytes found in semen are granulocyes [2].

Several studies have found that infertile men have a raised incidence of leukocytopspermia compared with their fertile counterparts [48–58]. In addition, leukocytospermia is associated with a significant reduction in sperm total numbers, motility, normal forms, and seminal fructose [26, 48–58]. Fructose is a marker of seminal vesicle function. These findings have not been replicated in all studies [59]. Leukocytospermia has also been implicated in a reduction in sperm penetration assays and pregnancy outcomes [60, 61].

Oxidative Stress and Reactive Oxygen Species

Oxidative stress is a pathophysiological process mediated by oxygen and oxygen derivatives called reactive oxygen species (ROS) [2]. ROS are part of normal cellular responses to destroy toxins such as infectious agents [43]. The human body has several strategies for protecting itself from damage that may be induced by ROS [43]. ROS injury occurs when these strategies are overwhelmed [43]. ROS-mediated sperm injury may be implicated in 30–80% of cases of idiopathic infertility [2, 3, 6, 43, 62]. Elevated ROS levels have been demonstrated in the

Table 6.4 Detection of leukocytospermia [2]

Histology/morphology	• Round cell counts • Bryan-Leishman stain • Peroxidase/Endtz test
Immunological techniques	• Immunohistology (gold standard) • Flow cytometry
Products of inflammation	• Elastase – <250 ng/mL: no inflammation – 250–1000 ng/mL: intermediate range – >1000 ng/mL: genital tract inflammation
Products of inflammation	• Chemiluminescence

Table 6.5 Indicators of oxidative stress from semen analysis and in vitro fertilization (IVF) [43]

Indicators of oxidative stress
Poor motility
Teratozoospermia
High number of round cells
Increased viscosity
Poor membrane integrity on hypo-osmolar swelling test (HOST)
Poor motility after overnight incubation with oocyte
Poor blastocyst development in absence of clear female factor
Poor fertilization on routine IVF

semen of 25–40% of infertile men [63, 64]; this rises to 90% in men with spinal cord injury (SCI) [65, 66].

There are 2 main sources of ROS generation within the semen:

- Spermatozoa
- Seminal leukocytes [2, 6, 43, 62].

Oxidative stress causes lipid peroxidation of the sperm membrane with a resultant abnormality of membrane composition, sperm motility, leukocytospermia, DNA fragmentation, and fertilization ability (Table 6.5) [2, 6, 43, 62]. Sperm, especially immature sperm, are highly vulnerable to oxidative injury as well as producing more ROS [2, 6, 43, 62]. Sperm are most vulnerable during transit through the epididymis where they must rely on their own intrinsic antioxidant capacity and that of the epididymis and testis, as they are not in contact with the seminal plasma antioxidants [43]. Seminal plasma has endogenous protective enzymes and antioxidants, which are deficient in infertile men [2, 43].

DNA Fragmentation

DNA damage may occur as a result of oxidative damage resulting in a reduction in genetic viability of spermatozoa [2]. This is associated with leukocytospermia, raised ROS, and poor semen parameters [2, 6]. Up to 8% of subfertile men with normal semen parameters may have high DNA fragmentation (Table 6.6) [6]. High

Table 6.6 Methods of measuring DNA damage [6]

Direct methods	• Single cell electrophoresis (COMET) • Terminal deoxynucleotidyl transferase medicated 2-deoxyundine 5-triphosphate (TUNEL)
Indirect methods	• Sperm chromatin structure assay (SCSA) • DNA intercalating dyes (acridine orange)

levels of DNA fragmentation (>30%) are associated with poor intrauterine insemination (IUI) and in vitro fertilization (IVF) outcomes [6].

Anti-sperm Antibodies

Anti-sperm antibodies (ASA) are present in 3–12% of men being evaluated for infertility [2]. Their production may arise from:

- Breakdown of blood–testis barrier,
- Inoculation of host with sperm antigens,
- Failure of immunosuppression [2].

Infection of the genitourinary tract is a risk factor for the development of ASA [2]. It has been postulated that ASA affects fertility by the agglutination of sperm, prevention of cervical mucous penetration, and the inhibition of the oocyte–sperm fusion [2]. However, there are conflicting reports in the literature about the association between leukocytospermia and ASA [2].

Treatment of Inflammation

Antibiotics

The goal of antibiotics administration is to treat infection. However, their role in restoring semen quality remains unclear. In addition, several antibiotic groups have been reported to adversely affect fertility [2].

Several studies have confirmed that men treated with antibiotics for a variable amount of time may have an improvement in pregnancy rates, sperm concentration, sperm motility, and a reduction in seminal leukocytes and ROS [43, 67, 68]. However, many of these studies have been criticized for their poor methodology [2]. Other studies have demonstrated reduced seminal leukocytes but no change in semen parameters [53, 69].

Antioxidants

As we have demonstrated that increased oxidative stress has a role in infertility, it would be sensible to expect that the use of antioxidants may be beneficial. A prospective study by Vicari et al. randomized men with infertility and leukocytospermia to 1 of 4 groups where they received nonsteroidal anti-inflammatory drugs (NSAIDs) and/or carnitine for 2–4 months [43, 70]. Treatment resulted in a

Table 6.7 Treatment of inflammation [2, 43]

Treatment of inflammation
Lifestyle factors • Smoking • Illicit drugs • Weight loss • Abstinence from alcohol
Minimize environmental exposure • Heat • Pollutants • Toxins
Treat underlying cause, i.e., antibiotics
Antioxidants
COX-2 inhibitors
Antihistamines

significant improvement in sperm vitality and reduction in seminal leukocytes [43, 70]. The group receiving NSAIDs for 2 months followed by 2 months of carnitine had the largest changes observed, with a significant increase in forward motility, the highest reduction in ROS, and the greatest pregnancy rate [43, 70].

COX-2 Inhibitors and Antihistamines

The evidence for the use of COX-2 inhibitors and antihistamines is much more limited, although there is some data to suggest that both may improve semen parameters in different ways [43, 71]. In addition COX-2 inhibitors may also reduce seminal leukocytes (Table 6.7) [43, 71].

Conclusion

In this chapter, we have discussed the most common organisms and their methods of causing pathology within the male reproductive tract. This is a topic that is often surrounded by controversy and conflicting evidence. The organisms that are probably most important are *C. trachomatis* and mumps.

Chlamydia causes significant disease throughout the male genital tract with a possible additional role in causing prostatitis. This is further compounded by the carrier often being asymptomatic, the high reinfection rates, and the lack of vaccine. It adversely affects semen parameters and may cause structural abnormalities resulting in obstructive azoospermia.

Paramyxovirus, which causes mumps, is another organism worth mentioning. We are now observing a resurgence in its incidence following a fall in public confidence in the vaccine. This is another organism that causes significant

pathology as well as affecting both hormonal production and semen parameters. These adverse effects may last for a significant period of time and in a small proportion may result in permanent infertility. The only management strategy for this condition is supportive and sustained reeducation of the public about the benefits of the vaccine.

Inflammatory markers may be indicators of previous subclinical infection as well as injury to the genital tract, which may be responsible for infertility of unknown cause. The treatment remains very controversial but we must at least educate our patients in reducing their lifestyle risk factors and treating any overt infections. It is important to have a frank discussion with our patients about the use of more controversial treatments such as further antibiotics and anti-inflammatories, especially prior to assisted reproduction.

In summary, this is an area that still needs more research. However, recognition and treatment of infections early remains important. During evaluation of the infertile male, the role of present or past infection must be discussed rigorously.

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Chapter 7
Ejaculatory Dysfunction: Retrograde Ejaculation

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Introduction

Retrograde ejaculation may be defined as a form of ejaculatory dysfunction in which seminal fluid is propelled proximally toward the bladder after leaving the ejaculatory ducts [1]. This process can be complete, in which case no semen is propelled in the antegrade direction, or partial, in which there is still some antegrade propulsion present. Premature ejaculation, anorgasmia, aspermia, lack of emission, and anejaculation accompany retrograde ejaculation as entities within the umbrella of ejaculatory dysfunction [1]. Depending on the series, retrograde ejaculation has been reported to be present in 0.4–2% of cases of male factor infertility [2, 3]. Although uncommon, retrograde ejaculation represents one of the few pathologies within the field of male infertility that, in select cases, can be successfully treated medically.

Clinical Vignette

A 30-year-old man presents to clinic with primary infertility. His wife is 25 years old, healthy, and already has had a child (now 2 years old) with a prior partner. She was evaluated by her reproductive endocrinologist and was found to have no

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abnormalities. The couple has been unable to conceive despite sexual intercourse 3–4 times per week. He reports that over the last 2–3 years, the volume of his ejaculate has been decreasing. He denies any other bothersome symptoms. He does not complain of anorgasmia, erectile dysfunction, low sex drive, depression, or fatigue. He does not have hematuria.

His past medical history is notable for type 2 diabetes mellitus diagnosed 6 years ago. He reports no history of prior surgery. His only medication is metformin. There is no family history of note. On physical examination, the patient is obese with a body mass index (BMI) of 38. His secondary sex characteristics appear appropriate. The penis is normal with an orthotopic urethral meatus and no evidence of hypospadias. Both testes are descended and normal in volume with no palpable masses. Both vasa are palpable. Digital rectal examination reveals normal sphincter tone and an appropriately sized prostate. No nodules or cystic structures are palpated. The review of his recent laboratory tests reveals glycosuria and a hemoglobin A1c of 12%. The patient relays that his primary care provider's office staff has contacted him about the laboratory results and he has an upcoming appointment with his primary care physician.

A semen analysis is ordered. The patient returns to clinic 10 days later to review the results. Ejaculate volume is shown to be 0.5 mL, with a sperm concentration of 10 million/mL and a total sperm count of 5 million. Sperm morphology and motility are normal. In accordance with American Society for Reproductive Medicine (ASRM) guidelines, a post-ejaculatory urinalysis is obtained. The results demonstrate >15 million sperm per mL in the collected sample.

The patient is started on oral pseudoephedrine. He tolerates this treatment well and blood pressure remains within normal limits during subsequent follow-up visits. While the patient admits to poor adherence to his metformin regimen in the past, he states that his desire to have a child has motivated him to get his diabetes under control. During the next several weeks, the patient begins to notice increased ejaculate volume. He is advised to continue regular timed sexual intercourse with his wife while on pseudophedrine. Three months later, he returns to clinic with news that his wife has recently become pregnant.

Pathophysiology

Ejaculation is composed of two phases: emission and expulsion. Emission is governed by sympathetic and parasympathetic discharge, which triggers a sequence of events causing seminal fluid to be discharged from the seminal vesicles and prostate. The first step is closure of the bladder neck, an action which is mediated by $\alpha(\text{alpha})_1$ adrenergic receptors. Prostatic secretions followed by seminal vesicle fluid and Cowper's and periurethral gland fluid are subsequently released through the ejaculatory duct and into the prostatic urethra [4].

Expulsion is governed by somatic innervation arising from the S2-4 nerve roots (Fig. 7.1). The motor branch of the pudendal nerve elicits coordinated contraction

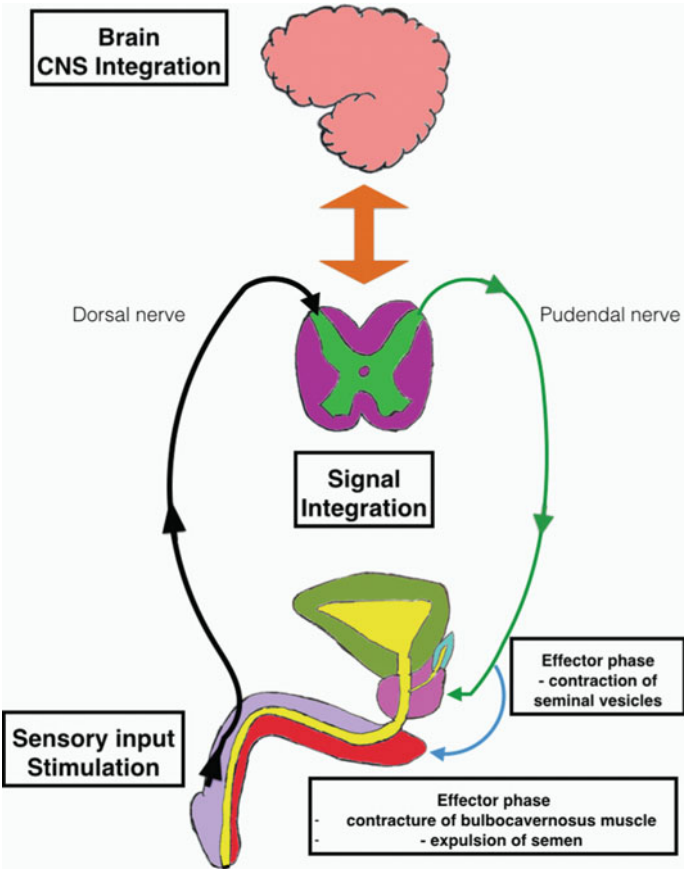

Fig. 7.1 Neural pathways involved in ejaculation

of the bulbocavernosus and bulbospongiosus muscles and nearby pelvic floor muscles. The external urinary sphincter relaxes, permitting antegrade expulsion of seminal fluid. The final factor permitting successful expulsion is the lack of anatomic obstruction.

While retrograde ejaculation is classically attributed to failure of bladder neck coaptation, there has been evidence that this is not the causative process in many cases. A study comparing transrectal color Doppler ultrasonography findings during ejaculation in a healthy man and a spinal cord-injured man demonstrated that in both patients, the bladder neck closed appropriately during ejaculation. However, the external urethral sphincter did not relax sufficiently to allow antegrade flow of semen in the spinal cord-injured man. Once the expulsion phase concluded, the semen in the prostatic urethra slowly flowed retrograde into the bladder [5]. As discussed in further detail in this chapter, α(alpha)-antagonist mediated ejaculatory

dysfunction has been shown to be an anejaculation caused by blockade of $\alpha(\text{alpha})_{1A}$ receptors in the vas deferens, prostate, and seminal vesicles.

In vitro studies have demonstrated that once exposed to the high osmolarity and acidic environment of the urine, spermatozoa lose motility and die quickly [6–8]. These findings underpin the importance of sperm preparation in the sperm retrieval techniques discussed in this chapter.

Diagnosis and Workup

A thorough history and physical examination are crucial to the diagnosis of retrograde ejaculation. Symptoms include decreased ejaculate volume or absent ejaculate as well as cloudy urine. In line with the common causes of retrograde ejaculation, affected men may have comorbidities that can result in neurogenic retrograde ejaculation such as diabetes mellitus or multiple sclerosis or may have had prior surgery that can affect the ability of the bladder neck to coapt. It is important to note that men with isolated retrograde ejaculation still retain the ability to orgasm. As discussed elsewhere in this text, orgasm and ejaculation are separate processes and inability to orgasm does not necessarily cause anejaculation or retrograde ejaculation or vice versa. However, the two may coexist.

Apart from neurologic examination findings associated with comorbidities such as diabetes mellitus or multiple sclerosis, men with isolated retrograde ejaculation tend to have otherwise normal genitourinary examination findings. In some men, the urine may appear cloudy due to the presence of semen. In addition, semen analysis may reveal a low-volume or absent ejaculate with or without oligospermia or azoospermia. In men with these semen analysis findings, obstructive azoospermia remains part of the differential diagnosis. Physical examination therefore represents an important opportunity for the clinician to distinguish between these disease entities. In a man with isolated retrograde ejaculation, both vasa deferentia should be palpable and the digital rectal examination should not reveal any palpable dilated midline structures, which are suggestive of ejaculatory duct obstruction.

The American Society for Reproductive Medicine (ASRM) recommends post-ejaculatory urinalysis for men with ejaculate volume <1.0 mL unless the patient is diagnosed with hypogonadism or congenital bilateral absence of the vas deferens [9]. However, there have been no strict criteria established defining a "normal" versus an "abnormal" post-ejaculatory urinalysis that is suspicious for retrograde ejaculation. Prior investigators have demonstrated the absence of sperm in the pre-ejaculatory urinalysis and the presence of sperm in the urine in post-ejaculatory urinalysis of fertile men. This supports the hypothesis that even in men without retrograde ejaculation, post-ejaculatory urinalyses may contain residual sperm that were washed out from the urethra during micturition [10]. Subsequent efforts have been made to refine the interpretation of post-ejaculatory urinalysis findings. A prospective study comparing 77 men being evaluated for infertility and 71 control male subjects by Mehta et al. showed that although the

presence of sperm in the post-ejaculatory urinalysis was more common in the group being evaluated for infertility, it was still very common even in the control group (98.7% vs. 88.7%, respectively). Subjects who were being evaluated for infertility were more likely to have greater than 50% of their total sperm count in the post-ejaculatory urinalysis and had a higher percentage of their post-ejaculatory urinary sperm count in the initial fraction of voided urine than were control subjects [11]. A similar study of 15 fertile men and 66 non-azoospermic men being evaluated for infertility found that 73% and 65% of the fertile and infertile men, respectively, had sperm in their post-ejaculatory urinalysis. This study did not find a statistically significant difference in proportion of sperm in urine between the two groups [12].

As previous studies demonstrate, there are no physical examination or diagnostic test findings that are pathognomonic for retrograde ejaculation. Findings can overlap considerably between fertile and infertile men. It is important to distinguish retrograde ejaculation from obstructive azoospermia and anejaculation, all three of which may present in a similar manner as azoospermia. Thus, diagnosis relies upon clinical suspicion combined with supportive findings on workup.

Etiologies of Retrograde Ejaculation

Neuropathic Changes

Genitourinary neuropathic diseases such as diabetes mellitus [13] and multiple sclerosis [14] have the potential to cause retrograde ejaculation (Table 7.1). Of these, diabetes mellitus is the most common cause of retrograde ejaculation, with various series reporting prevalence rates of 6–34.6% among diabetic men [15–18]. Of note, these figures may represent an underestimation of the true rate, as retrograde ejaculation among diabetics is felt by some investigators to be

Table 7.1 Etiologies of retrograde ejaculation

Neuropathic	Diabetes mellitus Multiple sclerosis Spinal cord injury
Postsurgical	Transurethral resection of prostate Photovaporization of prostate Transurethral microwave therapy of prostate Transurethral incision of prostate Retroperitoneal lymph node dissection Pelvic/spinal surgery
Medication-induced[a]	Antipsychotics?

[a]α(alpha)-antagonists have been shown to cause anejaculation, not retrograde ejaculation as is widely believed. Antipsychotics may cause ejaculatory dysfunction, but it is not clear whether this represents retrograde or anejaculation

under-diagnosed and underreported [19]. Cases of retrograde ejaculation in this patient population are thought to be caused by an autonomic neuropathy resulting in failure of bladder neck coaptation during ejaculation [20]. As with other sequelae of diabetes mellitus, the presence of ejaculatory dysfunction is correlated with the degree of the particular patient's glycemic control and the duration of diabetes. Theoretically speaking, retrograde ejaculation in patients with multiple sclerosis may be caused by the same mechanism as in diabetic patients, though the authors are not aware of any studies specifically demonstrating this mechanism. Prevalence estimates for retrograde ejaculation specifically are difficult to find in the literature. However, it has been reported that 31–50% of multiple sclerosis patients experience some sort of ejaculatory or orgasmic disturbance [21, 22].

Postsurgical

Postsurgical causes of retrograde ejaculation remain a common cause seen in urologic clinical practice (Table 7.1). Classically, surgical treatment for benign prostatic hypertrophy (BPH) has been long associated with retrograde ejaculation. The other prominent urologic cause of postsurgical retrograde ejaculation is patients undergoing retroperitoneal lymph node dissection (RPLND) for testicular cancer.

Transurethral resection of prostate (TURP) remains the gold standard for the surgical treatment of BPH. A thorough resection leads to a wide-mouthed TUR defect, causing failure of bladder neck coaptation. Patients should be counseled extensively preoperatively that retrograde ejaculation is very common after TURP. A recent review by Marra et al. noted that the overall rate of retrograde ejaculation was 66.1% [23].

Other treatment modalities for BPH have been examined with the goal of achieving comparable clinical efficacy compared to TURP while minimizing the side effects. Photovaporization of the prostate (PVP) was associated with a post-operative rate of retrograde ejaculation of 41.9% in 1 meta-analysis, compared to 61.4% in the TURP group. Less invasive techniques have lower reported rates of retrograde ejaculation. Examples include transurethral needle ablation (TUNA—0%), transurethral microwave therapy (TUMT—21.2%), and transurethral incision of the prostate (TUIP—21.1%) [23]. However, it must be noted that these techniques must be used on appropriately selected patients with smaller prostates.

The prostatic urethral lift procedure (UroLift®, Neotract, Pleasanton, CA) is a promising tissue-sparing technique where intraprostatic implants are delivered to separate the lateral lobes of the prostate without impacting the bladder neck. This offers relief of urinary obstruction without vaporization, ablation, or resection of prostatic tissue. Prospective studies have shown this technique preserves sexual function with no differences in preoperative and postoperative rates of sexual bother or ejaculatory dysfunction. Again, patient selection is important as the UroLift® technique has best results in patients with no median lobe, lack of an elevated bladder neck, and a post void residual of <350 ml [23, 24].

Retroperitoneal lymph node dissection disrupts the pre-aortic postganglionic sympathetic fibers, which can interfere with ejaculation (but not erection) [25]. Classically, reported rates of retrograde ejaculation after bilateral RPLND varied widely, with some series reporting rates of up to 90% [26]. However, recent advances in the understanding of the anatomy and physiology of erection, the adoption of modified unilateral templates of resection (sparing the contralateral sympathetic nerves, and use of nerve-sparing in bilateral templates) have decreased the risk of retrograde ejaculation after RPLND. A more recent series by Beck et al. showed preservation of normal ejaculation in 97% of patients undergoing primary RPLND [27]. Even patients undergoing post-chemotherapy RPLND had antegrade ejaculation rates of up to 85% [28].

Medication Side Effects

α(Alpha)-Antagonists

Alpha-antagonist medications such as tamsulosin, which are widely used for the treatment of lower urinary tract symptoms secondary to benign prostatic enlargement, are well known for their side effect of ejaculatory dysfunction (Table 7.2). Traditional teaching holds that the use of α(alpha)-antagonists causes loss of bladder neck coaptation, resulting in retrograde ejaculation. Over the last decade, multiple studies have demonstrated that this is, in fact, not the cause of α(alpha)-antagonist mediated ejaculatory dysfunction and that this process is actually mediated by antagonism of the $\alpha(\text{alpha})_{1A}$ adrenoreceptor.

Activation of this receptor is required for contraction of the vas deferens [29]. A study involving the use of real-time polymerase chain reaction on radical prostatectomy and cystoprostatectomy specimens showed that $\alpha(\text{alpha})_{1A}$ was also the predominant adrenoreceptor subtype in the seminal vesicles [30], another structure whose function is crucial to the process of ejaculation. The same study also assigned 17 healthy men to treatment with tamsulosin (predominantly $\alpha(\text{alpha})_{1A}$ vs. $\alpha(\text{alpha})_{1D}$ antagonism) and naftopidil (predominantly $\alpha(\text{alpha})_{1D}$ vs. $\alpha(\text{alpha})_{1A}$ antagonism) in a crossover manner. Results demonstrated lack of retrograde ejaculation and lack of sperm in post-ejaculatory urinalysis on transrectal color Doppler ultrasound after administration of either medication [30]. A randomized crossover study of tamsulosin, alfuzosin, and placebo in healthy adult males showed that the use of tamsulosin was associated with a >20% decrease in ejaculate volume in 90% of subjects and anejaculation in 35% of subjects in

Table 7.2 Medications associated with ejaculatory dysfunction

Medication class	Examples
α(alpha)-blockers	Tamsulosin, sildosin, doxazosin, prazosin, terazosin, alfuzosin
Antipsychotics	Levemepromazine, iloperidone, clozapine, risperidone

comparison with alfuzosin and placebo. In this study, there were no statistically significant differences in post-ejaculatory urine sperm concentrations between the three groups [31]. A randomized double-blind crossover study of 15 healthy male urologists demonstrated anejaculation in all subjects after 3 days of silodosin administration, with return of semen volume and sperm count to baseline 3 days after cessation of silodosin [32]. Interestingly, there has been speculation that the presence of ejaculatory dysfunction may serve as a sign of effective treatment of lower urinary tract symptoms in patients taking α(alpha)-antagonists for benign prostatic hyperplasia. In one literature review, an odds ratio of 1.68 was calculated for improvement in International Prostate Symptom Score by 3 points and maximal urinary flow rate by 3 mL per second [33].

Antipsychotic Medications

Antipsychotic medications used for the treatment of psychiatric disorders such as schizophrenia have been associated with rare reports of retrograde ejaculation (Table 7.2). Case reports have implicated several typical and atypical antipsychotics including levomepromazine [34], iloperidone [35], clozapine [36], and risperidone [37–39]—all of which have $\alpha(\text{alpha})_1$-antagonistic effects. The authors are not aware of any studies in which post-ejaculatory urinalyses were collected in patients taking these medications. It is therefore possible, as is the case with reports involving $\alpha(\text{alpha})_1$-antagonists, that these antipsychotics may be causing anejaculation instead of retrograde ejaculation. Alternatively, some investigators have suggested that ejaculatory dysfunction, retrograde or otherwise, may be the indirect result of other endocrine imbalances such as hyperprolactinemia which can be caused by these antipsychotics [40].

Spinal Cord Injury

Retrograde ejaculation has been diagnosed in men with spinal cord injury through the use of penile vibratory stimulation and rectal probe electroejaculation followed by catheterized post-ejaculatory urinalyses [41–43]. Certainly, there are known ejaculatory derangements that other investigators have proposed to be the cause of retrograde ejaculation in men with spinal cord injury. These include the tendency for sacral cord injuries to result in loss of the expulsion phase of ejaculation as well as detrusor-sphincter and detrusor-bladder neck dyssynergia [44–46]. However, as noted previously, post-ejaculatory urinalyses containing sperm are found even in neurologically intact men. Studies clearly demonstrating a mechanism of retrograde ejaculation in spinal cord-injured men remain relatively rare, with a notable exception being a study of nine men undergoing penile vibratory stimulation or electroejaculation with simultaneous urodynamic catheter monitoring of internal

and external urethral sphincter pressures. This study demonstrated that in each of the nine men, forceful contraction of the external followed by the internal urethral sphincter preceded ejaculation, but that in the men undergoing electroejaculation, the external sphincter pressure exceeded that of the internal sphincter shortly after ejaculation, providing a possible mechanism for electroejaculation-induced retrograde ejaculation [47]. As mentioned earlier, transrectal color Doppler ultrasonography studies have shown that the bladder neck or internal sphincter closes appropriately during ejaculation in some patients with spinal cord injury. Rather, it is a failure of external urethral sphincter relaxation, or a form of detrusor-sphincter dyssynergia, that causes retrograde ejaculation in these patients [5]. While rare, spinal cord conditions such as perineural cysts or tethered cord syndrome can cause retrograde ejaculation as well [48, 49].

Treatment of Retrograde Ejaculation

Noninvasive Techniques

The method of treatment of retrograde ejaculation can vary widely depending on severity and etiology (Table 7.3). In general, less invasive treatment options are preferred for initial management. In some cases simple substitution, discontinuation, or dose reduction of the offending medication can restore normal ejaculation. In cases of retrograde ejaculation caused by antipsychotic medication, referral to a psychiatrist for dosage adjustment or substitution with a different medication may be indicated. Dose reduction has been reported to be successful in restoring normal ejaculation in patients taking risperidone, for example [38]. Prior authors have reported on circumvention of retrograde ejaculation by instructing men to ejaculate with a full bladder. According to one published case report, two patients with retrograde ejaculation were able to convert to antegrade ejaculation with the use of this technique [7]. In a separate report, ejaculate retrieved in this manner from one

Table 7.3 Treatment of retrograde ejaculation

Noninvasive	Discontinuation, dose reduction of medications	Ejaculation with full bladder	
Medical	α(alpha)-agonists (midodrine, pseudophedrine, ephedrine)	Anticholinergics, antihistamines, tricyclic antidepressants	
Sperm retrieval	Modified Hotchkiss technique	Retrograde instillation of sperm media and extraction	Epididymal or testicular sperm extraction
Surgical	Young-Dees technique (bladder neck reconstruction)	Y-V plasty of bladder neck	Transurethral collagen injection of bladder neck

patient was used for intrauterine insemination and resulted in a successful pregnancy [50]. Other noninvasive techniques include the combination of urinary alkalinization followed by voiding of urine directly into the vagina after intercourse or intravaginal insemination of the postcoital urine–semen mixture using a syringe [51–53].

Medical Therapy

Medical therapy has been described in the literature, mostly in the form of α(alpha)-agonists, tricyclic antidepressants, and antihistamines aimed at increasing sympathetic tone and/or decreasing the parasympathetic tone of the bladder neck. Commonly used α(alpha)-agonists include midodrine (known as milodrin in several older studies), pseudoephedrine, and ephedrine. The use of these medications has been described in the past [54, 55]. A few notable recent studies involving patients diagnosed with retrograde ejaculation have been published. A study of 33 patients with diabetes mellitus and retrograde ejaculation found that imipramine, pseudoephedrine, and a combination of both were able to produce antegrade ejaculation in 38.5, 47.8, and 61.5% of cases, respectively [56]. Hsiao et al. were able to restore antegrade ejaculation using pseudoephedrine in two of four patients with retrograde ejaculation after chemotherapy and retroperitoneal lymph node dissection for testicular cancer [57]. Tomasi et al. reported on the use of intramuscular self-injection of methoxamine 30 min prior to sexual intercourse or masturbation in two patients. Antegrade ejaculation was restored in both patients with one of the two patients able to achieve pregnancy after 3 months of methoxamine treatment [58].

Tricyclic antidepressants also have been used for the medical treatment of retrograde ejaculation. In one series, 11 of 11 patients with retrograde ejaculation after retroperitoneal surgery were treated with daily imipramine for 7 days prior to the female partner's planned ovulation. Using this regimen, antegrade ejaculation was produced in all 11 patients with only minor side effects such as dizziness, weakness, nausea, or sweating. Two spontaneous pregnancies were reported and two successful fertilizations without successful pregnancy were induced with the help of intracytoplasmic sperm injection (ICSI) [59]. In another study, antegrade ejaculation was achieved in three of seven patients using imipramine. Of these three patients, two went on to conceive naturally. All three of these patients initially had partial retrograde ejaculation [2]. The use of various other medications has been described in some older studies including the antihistamines bromphenoramine [60] and chlorpheniramine—the latter in combination with phenylpropanalamine [55].

A meta-analysis reported that of retrograde ejaculation patients treated with medical therapy, antegrade ejaculation was achieved in 50% and spontaneous pregnancy was achieved in 34%. The authors found that imipramine was the most widely used medication but did note that there were no randomized controlled trials comparing the various medical treatments of retrograde ejaculation on a head-to-head basis [55]. Indeed, some clinicians prefer the use of alpha-agonists

such as pseudoephedrine due to the potentially more dangerous adverse effects associated with imipramine [57].

Sperm Retrieval

In cases of retrograde ejaculation that persist despite the aforementioned interventions, sperm retrieval therapy may be used. Sperm retrieval may also be accomplished by a multistep process known as the modified Hotchkiss technique. The initial step involves the use of agents to make the urine osmolality and pH more favorable to sperm motility and viability. Some investigators have accomplished this by first asking the patient to empty his bladder and then catheterizing the bladder and instilling an alkalinizing medium. Others have favored the use of oral sodium bicarbonate and hydration. The patient is then asked to empty his bladder prior to any ejaculation attempts. Masturbation or, if necessary, penile vibratory stimulation or electroejaculation is then used to produce retrograde ejaculation into the previously instilled medium or the alkalinized urine. The sperm are retrieved either by voiding or bladder catheterization. The urine is centrifuged and the sperm pellet is resuspended in a buffered medium. The suspension is then used for intravaginal insemination or assisted reproductive techniques such as intrauterine insemination, in vitro fertilization, and ICSI. Many variations on this technique have been described, ranging from the use of unprepared urine and sperm to centrifugation and suspension of the sperm within media such as Ham's F-10 medium, bovine serum albumin, human serum albumin, and phosphate buffered solution [2, 51, 61–64]. Prior investigators who have reported successful pregnancies with sperm retrieval were able to alkalinize the urine to a "physiological" pH of 7.2 [65].

Generally, this process is timed in accordance with the partner's luteinizing hormone (LH) surge in order to maximize the probability of success. However, there are at least two published reports of successful pregnancy or live birth via ICSI using thawed sperm that were initially retrieved by a modified Hotchkiss technique and then cryopreserved for a period of time [66, 67]. One recent review of 15 published articles using modified Hotchkiss techniques dating back to the 1970s calculated a pregnancy rate of 15% per cycle [68].

Surgical Therapy

Surgical correction of retrograde ejaculation has been described sparingly in the literature, though with a high rate of success in the limited number of cases reported. The Young-Dees technique of bladder neck reconstruction, which has been used for the management of incompetent bladder necks due to trauma, neurogenic bladder, and exstrophy, has also been applied to patients with retrograde

ejaculation. One series reported the successful conversion of retrograde into antegrade ejaculation in four of five patients [69]. Using a similar technique, Ramadan et al. described the use of trigonal urothelium and musculature in order to reconstruct the proximal urethra and bladder neck around a 12-French catheter. This technique was used in five men with retrograde ejaculation after bladder neck surgery for bilharzial obstruction, also with restoration of antegrade ejaculation in four of the five men [70]. A third publication reported successful correction of retrograde ejaculation in two men who developed retrograde ejaculation after Y-V plasty of the bladder neck. Normal ejaculation was achieved in both patients and one patient was able to produce a successful pregnancy [71].

A less invasive option in the form of transurethral collagen injection of the bladder neck has been used for the treatment of retrograde ejaculation. A prospective trial of 23 patients with type 1 diabetes mellitus randomized to transurethral collagen injection of the bladder neck or a sham surgery demonstrated statistically significant improvements in antegrade ejaculate volume, sperm count, motility, and progressive motility in the treatment group at 1 year postoperatively. There were no complications reported and no significant differences in erectile function as measured by International Index of Erectile Function-5 questionnaire scores. Interestingly, the patients undergoing collagen injection demonstrated improvements in depression and anxiety as measured by validated questionnaires [72]. A case report from Japan demonstrated the use of this technique in a 40-year-old male with retrograde ejaculation following thoracic spinal cord injury. Preoperatively, his retrograde ejaculation was documented by transrectal Doppler ultrasound. He then underwent transurethral collagen injection of his bladder neck. At 14 days postoperatively, repeat ultrasound demonstrated the conversion of his retrograde ejaculation to antegrade ejaculation. The therapeutic effect of the collagen had disappeared at 1 year postoperatively, but the injection was repeated, again with successful restoration of antegrade ejaculation [73]. A similar outcome was observed following bladder neck collagen injection in a 36-year-old man who presented with primary infertility refractory to pseudoephedrine therapy. He had had a history of Y-V plasty of the bladder neck, a surgical method of bladder neck obstruction in which a Y-shaped incision is made through the area of obstruction and the apex of the V flap is sutured to the Y incision. Successful pregnancy was achieved with intrauterine insemination 3 weeks postoperatively [74]. In these reported cases, the surgery seems to have been well tolerated. That said, due to their invasiveness and the risk of postoperative complications, the authors recommend that surgery should be considered as a last resort for the treatment of retrograde ejaculation.

When successful, the techniques discussed above merely make the patient's own sperm available for insemination but do not improve the quality of the sperm. Thus, in patients who produce poor quality sperm, these techniques may need to be bypassed and assisted reproductive techniques may be indicated. Case reports and case series describe the use of vasal aspiration and intrauterine insemination [75],

percutaneous epididymal sperm aspiration [76], and testicular sperm extraction and ICSI [57, 77] in patients with retrograde ejaculation, resulting in successful pregnancy.

Conclusion

Recent evidence suggests that some classical examples of retrograde ejaculation are in fact more appropriately categorized as examples of anejaculation. Furthermore, in many cases appropriately labeled as retrograde ejaculation, alternative causative mechanisms besides failure of bladder neck closure may be at fault. More so than certain other forms of male infertility, retrograde ejaculation represents an entity that can be successfully treated. Thus, these are important findings that deserve further study and may impact treatment options in the future. For patients with otherwise healthy sperm, as long as the proper neurologic and anatomic derangements are identified and effective treatment strategies can be implemented, successful conception should be within reach.

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Chapter 8
Management of Azoospermia

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Introduction

Azoospermia is defined as absence of spermatozoa in the ejaculate. It is found in around 15% of men presenting with infertility [1]. The introduction of intracytoplasmic sperm injection in the early 1990s was coupled with major advances in surgical sperm retrieval. This dramatically changed the concept in management of azoospermia patients giving them the hope of having their biological child.

Classification

In general, azoospermia is classified as non-obstructive (NOA) or obstructive (OA).

Non-obstructive Azoospermia

Non-obstructive azoospermia causes can be either pre-testicular (endocrinal) or testicular (testicular failure).

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Pre-testicular

Pre-testicular causes are due to hypothalamic and pituitary malfunction, which may be congenital hypogonadotrophic hypogonadism; the most frequent of which is Kallmann syndrome. Other rare congenital causes are isolated luteinizing hormone (LH) or follicle stimulating hormone (FSH) deficiency, Prader–Willi syndrome, Bradiet Biedel syndrome, and cerebellar ataxia [2]. Acquired causes may include pituitary tumors or infarcts, exposure to radiation, blood diseases such as hemochromatosis, where 17% of patients may suffer from testicular failure and manifest by hypogonadism [3], and sickle cell anemia due to micro-infarcts in the pituitary [4].

Hyperprolactinemia will lead to inhibition of testosterone production either directly at the testicular level or indirectly through inhibition of pituitary gonadotropins, which may eventually inhibit spermatogenesis leading to NOA [5].

Exogenous steroids exposure may be either due to steroid abuse, replacement therapy or occupational exposure. This will lead to negative feedback inhibition of pituitary gonadotropins leading to azoospermia.

Testicular

Testicular causes of NOA are idiopathic in about 49–93% of cases [2]. Genetic abnormalities may account for up to 21%; the most common is Klinefelter's syndrome followed by chromosomal translocations and Y-chromosome microdeletions in any of azoospermia factors (AZF) A, B, or C. Other rare genetic causes include XX male syndrome, XYY syndrome, vanishing testis syndrome, Noonan syndrome (Turner syndrome), and myotonic dystrophy [6, 7].

Testicular injury may lead to NOA if both testes are affected. Injury could be due to inflammation, orchitis, especially mumps orchitis or testicular trauma with significant loss of testicular tissue or testicular vascular damage (torsion and infarction).

Exposure to gonadotoxins may also lead to NOA especially chemotherapy and radiotherapy. Systemic diseases such as liver and renal failure can affect spermatogenesis leading to NOA [8, 9]. Bilateral undescended testes will usually present with NOA. Varicocele can cause azoospermia, however, this is controversial [10, 11].

Obstructive Azoospermia (Post-testicular)

Congenital seminal tract obstruction may occur at different levels. The most common is bilateral absence of vas deferens, which can be associated with complete or partial absence of epididymis, seminal vesicles, and ejaculatory duct (see Chap. 10). This is considered a subset of cystic fibrosis syndrome caused by

mutation in the CFTR gene. Other congenital causes for OA include Young syndrome, idiopathic epididymal obstruction, Von Hipple–Lindau, and cystic dysplasia of rete testis.

Congenital ejaculatory duct obstruction (EDO) is usually due to midline prostatic cysts originating from embryonic remnants of Wolfian or Müllerian ducts.

Acquired OA is usually a sequel to inflammation at the epididymal, vasal, or ejaculatory duct level. This may be caused by sexually transmitted diseases (STDs) (especially gonorrhea), tuberculosis, chronic prostatitis, or epididymitis.

Iatrogenic injury of seminal tract can happen during hydrocelectomy, hernia repair, or following vasectomy [12–15].

Case Scenario 1

Mr. A presented to the male infertility clinic after 1 year of marriage complaining of failure to conceive with his wife. The couple was very anxious after receiving semen analysis results showing absence of sperms.

The first step in dealing with these cases is to confirm the diagnosis of azoospermia [2, 16, 17]. Not every ejaculate is semen; some men will fail to reach orgasm and will be giving urethral secretions only. A very short abstinence period may give a false result, as well as spillage of the semen out of the collection container. This can be avoided by giving proper instructions for semen collection [18].

Proper semen analysis technique for azoospermia samples must include high-speed centrifugation up to 3000 xg [18] and thorough reassessment of the pellet to differentiate azoospermia form cryptozoospermia, which is defined by the presence of a very small number of live sperm in the ejaculate that may be detected only after centrifugation [19]. Temporary azoospermia can be seen following systemic or genital infections and can last for 2–3 months [20]. Therefore, more than 1 semen sample with proper timing should be done before confirming azoospermia diagnosis.

Following proper evaluation of Mr. A, his doctor diagnosed him as a case of NOA. How did he reach this diagnosis?

Clues Toward Diagnosis of Non-obstructive Azoospermia

History and Medical Examination

Certain clues in the past medical and surgical history may raise the possibility of NOA diagnosis. This may include past medical history of any testicular or pre-testicular cause; e.g., orchitis, cryptorchidism, or trauma, history of

gonadotoxin exposure, such as prior radiation therapy/chemotherapy, or recent fever or heat exposure [2–21].

Patients with pre-testicular causes will usually presents with symptoms suggestive of hypogonadism including delayed or failed puberty, low libido, erectile dysfunction, or low ejaculate volume. Anosmia, if present, will guide the diagnosis toward Kallman syndrome. Together with sexual dysfunction, visual field defect and manifestation of increased intracranial tension can be the presenting symptoms for hyperprolactinemia [22].

Family history taking can be helpful. Although there is a controversy regarding familial incidence of infertility, many studies have reported siblings affected with the same pattern of infertility [23–25]. A recent study had reported the occurrence of NOA in two siblings of seven families [25]. In each family, testicular histopathology and surgical sperm retrieval (SSR) result were the same for both siblings although no known genetic abnormality could be detected. So the cause in this case is most probably unknown point mutation in any of the genes regulating spermatogenesis. Therefore, positive consanguinity and family history of infertility in siblings will raise the possibility of underlying genetic cause [25].

Some occupations may have deleterious effect on semen and may even lead to azoospermia especially if it involves exposure to gonadotoxins, such as working in areas with increased radioactivity or excessive heat as in steel manufacture [26]. Another lifestyle factor that may affect testicular function is obesity. In a recent systemic review by Sermondade et al., an elevated risk of infertility was detected among couples when the male partner is overweight or obese. This observation is mainly due to a higher incidence of semen abnormalities among obese patients. Reasons for this association are likely to be related to obesity's effect on male reproductive hormones. Significant reductions in total testosterone, free testosterone, and sex hormone-binding globulin (SHBG) as well as elevations of estrogens in obese men are well documented [27, 28].

During general examination, lack of secondary sexual characteristics including sparse body and facial hair, and feminine body fat distribution is mainly seen in patients with pre-testicular and some cases of testicular causes of azoospermia.

Local genital examination may provide more clues to aid the diagnosis of NOA. Normal testicular volume is greater than 19 ml. Small testicular size may favor the diagnoses of NOA but it is nonconclusive. On the other hand, normal testicular size is frequently seen in cases with spermatogenic arrest and Sertoli cell only. Testicular consistency may help the diagnosis of testicular masses if palpable. The epididymis is expected to be of normal morphology and both vasa differentia are usually present. Presence, laterality, and grade of varicocele are part of the routine evaluation of an infertile male. Hypogonadism can also manifest with small penile size as well as small prostate size on digital rectal examination [17, 21].

Investigations

- **Semen analysis**—NOA will usually present with normal volume, fructose positive semen samples. However, severe hypogonadism may lead to low volume, fructose positive semen. Other causes of low semen volume may include retrograde ejaculation or ejaculatory duct obstruction associated with NOA [17].
- Advanced semen staining and testing techniques may help in the diagnosis of NOA like MGG stain, where shed spermatogenic cells may be identified in centrifuged semen sample [29].
- **Hormonal assessment**—According to the American Urological Association (AUA) guidelines, initial assessment of an azoospermia patient should include FSH and testosterone. If testosterone is low, LH and prolactin should be tested. Low FSH, LH, and testosterone confirm the diagnosis of hypogonadotrophic hypogonadism. Elevated FSH with or without low testosterone is an indicator of spermatogenic failure. On the contrary, presence of normal FSH and LH does not exclude NOA [17, 30].
- **Genetic testing**—It should not be done for every azoospermic patient and must be tailored to each case. It should not be done for men with strong suspicion for obstruction with normal semen volume and vas deferens palpable bilaterally, or in patients with known prior history of fertility or previously documented sperm concentration >5 million/mL of ejaculate fluid. Also, genetic testing is not indicated in patients with known prior toxic exposure such as cytotoxic chemotherapy or radiation [17, 18].
  Karyotyping is done to detect chromosomal abnormalities; e.g., gain or loss of a complete chromosome, inversion, or chromosomal rearrangements such as translocations.
  Y-chromosome microdeletion is present in 13% of NOA cases. AZF-a and-b carrier is the worst diagnosis with 0% chance to find sperm in testis while sperm could be retrieved by TESE in up to 70% of AZF-c deletion cases. Therefore, Y-chromosome microdeletions must be done for all cases of NOA to help in counseling the patients for SSR expected success [31, 32].
  Some specific genetic defects may be needed; e.g., Kal-1 gene mutations should be tested in suspected Kallmann syndrome cases [21].
- **Radiology**—In cases with hypogonadotrophic hypogonadism, magnetic resonance imaging (MRI) of the brain and sella should be done to rule out neoplastic lesions that may be infiltrating or compressing the hypothalamus or pituitary [21]. Similarly, MRI is recommended to diagnose pituitary micro- or macro-adenoma in cases of hyperprolactinemia [33].
  Ultrasound does not play an important role in diagnosis of NOA; however, scrotal ultrasound may help to diagnose testicular tumors because of their increased incidence in patients with infertility by 1.6–20 times more than fertile men [34]. It is also indicated in cases with abnormal findings during scrotal

examination, such as abnormal consistency or discrepancy in size between the two testes. It may also aid to confirm the diagnosis of varicocele. Pelvic ultrasound is needed to locate the site of the testis in cases of cryptorchidism. Finally, transrectal ultrasound (TRUS) may help in visualizing the small prostate size and seminal vesicles present in cases of severe hypogonadism [35].

- **Diagnostic testicular biopsy**—Testicular biopsy can show the level of testicular impairment but it cannot predict success of SSR. According to AUA best practice guidelines, diagnostic testicular biopsy can be done in patients with azoospermia with normal testicular size and normal or borderline elevated FSH to differentiate between obstructive and non-obstructive azoospermia cases. However, it is better to take a portion of the testicular tissue during the same procedure for possible cryopreservation if sperm are available for future use in in vitro fertilization (IVF). The biopsy can be unilateral or bilateral but if taken unilaterally, it should be from the larger side [17]. The testicular biopsy technique will be discussed later in this chapter.
- **Future advances in investigations**—Next-generation DNA sequencing may be helpful in detection of de novo gene mutations affecting fertility, especially in cases of familial or idiopathic NOA [36].
  Proteomics of semen may help in detection of unique spermatogenic markers in semen that may aid diagnosis and treatment of NOA cases [37, 38].

Management of Non-obstructive Azoospermia

The management of NOA is so challenging. Proper counseling of patients is essential to deliver the message that although this is a severe case of infertility, yet there is still good prognosis for parenting their own children. Studies have proven presence of small foci of spermatogenesis in 30–60% of NOA patients [39, 40]. Although the easiest way is to perform a trial of surgical sperm retrieval, it is better to adopt a stepwise protocol aiming to stimulate spermatogenesis with possibility of sperm appearing in semen or at least improve the success rate of SSR. This can be done through the following:

Optimizing Lifestyle Factors

- **Obesity**—Weight loss is generally believed to be of value in restoring fertility and normal hormonal profiles. Few studies confirmed a favorable effect for bariatric surgery in correcting hormonal imbalance and improving the sexual quality of life [41, 42]. Therefore, it is generally advised to reduce weight in patients with NOA whether surgically or nonsurgically before going for SSR.

- **Occupation**—If possible it is advised to change occupations that may affect testicular function, such as those associated with exposure to excessive heat (ovens, steel manufacture) or gonadotoxins (radiation, pesticides, cement, and heavy metals) [43].
- **Smoking**—Many studies have shown the deleterious effect of smoking on spermatogenesis as well as other body systems. Therefore, it is better advised to quit smoking to avoid its toxic effect [44].

Correction of Reversible Causes

- **Stoppage of causative medications**—Anabolic steroids abuse is the most common medication that leads to NOA due to the negative feedback on pituitary and hypothalamus. Immediate cessation of abuse is the first line of treatment in these cases. This may lead to reversal of negative feedback and appearance of sperm in semen in 84% of patients within 6 months [45]. Some cases may require stimulation of spermatogenesis using antiestrogens or gonadotropins. However, there are no studies on their effect in patients with anabolic steroid abuse.

  With the advancements in medical oncology, the life expectancy of cancer patients has improved and more patients will seek fertility later on in life. Cancer treatment modalities such as radiotherapy and chemotherapy lead to NOA, which can be reversible in 55–90% of patients within 3–5 years posttreatment, depending on the medication used, doses, and length of treatment. This necessitates fertility preservation prior to these treatment modalities, which can be done by cryopreservation of multiple semen samples for future use in IVF if NOA is persistent [46].
- **Specific medical treatment**—Hormonal replacement therapy in cases of hypogonadotrophic hypogonadism. These are the cases with the best prognosis to treatment with a successful appearance of sperm in semen and spontaneous pregnancy in 50–80%. Supplementation is usually done using FSH and human chorionic gonadotropin (HCG). Different treatment protocols are present but the most used is to start by intramuscular (IM) HCG 2000 IU 3 times or 2500 IU twice per week for 6 months to prime the testis for spermatogenesis. FSH 75–150 IU subcutaneously (SC) is added after 3–6 months. HCG can initiate spermatogenesis in some cases. Treatment should continue for at least 6 months up to 2 years before sperm appear in semen. Noncompliance with treatment protocol may lead to failure of treatment [47–49].

  Another method for supplementation is gonadotropin-releasing hormone (GnRH) administration (intravenous, subcutaneous, or intra-nasally). However, the bi-hourly administration makes it impractical and inconvenient for patients. GnRH can be reserved to cases resistant to HCG and FSH [50, 51].

In cases that remain azoospermic following hormonal treatment, sperm could be successfully extracted from the testis in around 73% of them.

- **Treatment of hyperprolactinemia**—If hyperprolactinemia is drug induced the best treatment option is stoppage of the causative medication if possible. Otherwise, dopamine agonists may be used. If hyperprolactinemia is secondary to prolactinoma, dopamine agonists are recommended. Cabergoline is preferred to other dopamine agonists because it has higher efficacy in normalizing prolactin levels, as well as a higher frequency of pituitary tumor shrinkage [33].
- **Surgical treatment (varicocelectomy and NOA)**—Azoospermia in association with a varicocele is estimated to range between approximately 4–14%. Recent studies have demonstrated improvements in semen parameters after varicocele repair in men with azoospermia. The primary benefit of varicocelectomy in men with NOA is the possibility of producing motile sperm in the ejaculate. Varicocele repairs in azoospermic men have also increased the success rates of assisted reproductive techniques such as intracytoplasmic sperm injection (ICSI) by providing motile sperm from fresh ejaculate or for testicular sperm extraction (TESE). Some studies report that varicocele repair using microsurgical technique in patients with azoospermia has resulted in approximately 40% improvement of semen parameters and the appearance of motile sperm in the ejaculate [52–55].
- **Empirical medical treatment**—The role of medical treatment in NOA is mostly empirical, however, the poor pituitary gonadal function in these patients warrants the chance of benefit from medical stimulation of spermatogenesis [9]. Hormone therapy has been generally accepted as a form of medical treatment for patients with NOA prior to assisted reproductive technology (ART). The rational for this is to increase intratesticular endogenous testosterone that would in its turn enhance spermatogenesis and improve SSR [56]. A tray of medications are available for treatment with varying and contradictory success rates according to different studies:
  - **Antiestrogens (clomiphine citrate and tamoxifen)**—The mechanism of action of these medications is to block estrogen receptors at hypothalamus and pituitary, preventing negative feedback of estrogen on gonadotropin secretion resulting in increased GnRH, FSH, LH, and resultant increase in serum testosterone. However, the effect on SRR is controversial. Hussein and coworker in 2005 reported appearance of sperm in semen in 64% of NOA cases included in their study and successful SRR in all cases that remained azoospermic. However, they excluded all cases of Sertoli cell only syndrome and small testicular size [57]. Again in 2012 the same group reported significant improvement in SRR in NOA patients following hormonal stimulation of spermatogenesis using clomiphene citrate alone or combined with HCG or FSH [58]. On the other hand, Reifsnyder et al. stated that although testosterone level was significantly increased after using antiestrogens in NOA patients, the SRR and live birth rates did not improve [56].

- **Aromatase inhibitors**—Alterations in testosterone/estrogen ratio negatively impacts spermatogenesis through decreased LH, FSH levels by direct feedback inhibition of HPG axis by the high estrogen level, which will subsequently inhibit androgen synthesis in testis and have direct adverse effect on spermatogenesis. Aromatase inhibitors block conversion of testosterone to estrogen, abolishing these negative impacts on spermatogenesis.
- In a recent study on **Klinefelter's syndrome** patients, the authors reported a significant improvement of SRR in patients using anastrozole—an aromatase inhibitor—over patients receiving other hormonal therapies for induction of spermatogenesis [59].
- **Gonadotropins**—While normal FSH and LH levels are needed to maintain active spermatogenesis, high levels of these hormones found in some NOA cases are believed to cause downregulation of FSH and LH receptors in Sertoli and Leydig cells. Some authors have suggested that a "gonadotrophin reset" is the only strategy to prevent this gonadotrophin hyperstimulation with subsequent improvement of spermatogenesis and SRR [60–62].

- **Surgical sperm retrieval and IVF**—Recent studies suggest that almost 60% of men with NOA have some sperm production in the testes [63]. Spermatogenesis in these cases is usually focal and defective with production of few numbers of sperm. These sperm can be used for intracytoplasmic sperm injection (ICSI). However, the fertilization and pregnancy rates following the use of testicular sperm in NOA patients are reduced.

  - **Genetic counseling**—Prior to surgical sperm retrieval, proper genetic counseling should be done and both couples should consent for the procedure as the presence of genetic abnormalities may not only affect the success rate of SSR but also affects the success rate of the ICSI trial itself.
    Carriers of balanced translocations, although phenotypically normal, are at risk of infertility, repeated miscarriages, and offspring with unbalanced karyotype [64, 65]. Reciprocal translocations patients possess more incidence of production of aneuploidic sperm than Robertsonian translocations [66, 67]. Preimplantation genetic diagnosis (PGD) for chromosomal translocation cases has been very successful in assisting couples to achieve a viable unaffected pregnancy and has certainly reduced the time taken to achieve a pregnancy by reducing the number of spontaneous abortions [68]. However, there is still a possibility of pregnancy with an unbalanced embryo, which may result in fertility problems for the child in future [69].
    Microdeletion of AZF-a or-b will always be associated with Sertoli cell only syndrome and no chance of sperm retrieval. However, patients with microdeletion of AZF-c have the same successful testicular SRR as other idiopathic cases of NOA [70].
    When sperm is available for ICSI in Yq microdeletion individuals, couples must be genetically counseled that Yq microdeletion will inevitably be

passed on to all of their male offspring, which will ultimately mean that all male offspring will have reduced infertility [69]. In these cases, transfer of only female embryos during ICSI is the best choice.

In cases of Klinefelter's syndrome, the SRR is similar to idiopathic NOA cases and may reach up to around 70% [56, 59, 71, 72]. Although Klinefelter syndrome patients have a high ability to produce aneuploid sperm, there appears to be some meiotic check points that eliminate these aneuploidic sperm. Less than 10% of the offsprings of these patients will have numerical chromosomal defect [73, 74]. These couples should be counseled and advised to go PGD to choose only normal embryos for transfer during ICSI.

- **Fresh or frozen sperm**—Given the possibility of not finding sperm in the testicles in up to 40% of NOA cases, patients usually ask about the timing of SSR. Is it better to do SSR on the same day of ICSI or perform it at an earlier date before ovulation induction for the spouse and cryopreserving the sperm if found to be used for future ICSI trial?

  This decision is very debatable, although there is evidence favoring simultaneous SSR and ICSI. Sometimes in NOA, sperm retrieved by SSR is very few to the extent that cryopreservation cannot be done. Moreover, the post-thaw survival of testicular sperm in NOA cases is low with significant decrease in sperm motility and vitality, which may hinder the use of these sperm in the following ICSI trial and necessitates repeat of SSR procedure [75]. The successful SSR rate in the second attempt is 80% [76]. Although this success rate is high, there is a 20% chance for the couple to lose the chance of ICSI trial and even hope of fertility.

  On the other hand, unnecessarily giving ovarian stimulation for the female in 40% of NOA cases with all the financial and psychological effects as well as the morbidity from this stimulation is still a point of debate.
- **Techniques of SSR**—There are many methods for surgical sperm retrieval with different SRR as well as benefits and drawbacks. Table 8.1 summarizes these techniques [72, 77–83].

- **Further management of NOA cases**—In cases where the patients decide not to go for SSR or with failure to find sperm during SSR, the patients are left with few choices for fertility. The first choice would be intrauterine insemination or ICSI using donor sperm. The other choice would be adoption.
- **Future management of NOA**—Stem cell therapy for male infertility is still in the very early investigational steps. We believe that in the near future there would be a solution to the fertility problem for most NOA cases using stem cells.

Table 8.1 Different surgical sperm retrieval techniques [72, 77–83]

	TESA	Mapping	TESE	Micro TESE
Sperm retrieval rate (%)	49.5	47	45	62
Advantages	Simple Can be repeated Less cost	Simple Gives a guide to subsequent biopsy	No microsurgical skills Less cost	High surgical sperm retrieval rate (SSRR) Lower incidence of hematoma Lower incidence of postoperative testicular atrophy
Disadvantages	Lower SSRR	Multiple punctures may increase incidence of hematoma Sperm found are not cryopreserved.	Lower SSRR than microTESE Higher incidence of hematoma and fibrosis Higher incidence of postoperative testicular atrophy Higher risk of decreased androgen production	Higher cost Requires microsurgical skills

TESA—testicular sperm aspiration, *TESE*—testicular sperm extraction

Case Scenario 2

Mr. M is a 34-year-old gentleman, married for 7 years, and has one child who is 5-years old. He and his wife have been trying to have a child for the past 3 years. The wife consulted a gynecologist who confirmed that she had no cause for infertility. He ordered a semen test for Mr. M and the result came as absence of sperm in the whole sample. He was referred to an urologist who diagnosed him as a case of obstructive azoospermia after proper evaluation.

Clues Toward Obstructive Azoospermia

History and Medical Examination

History of infection—whether urinary tract infection, prostatitis, epididymitis, or sexually transmitted diseases—may evoke the possibility of OA whether on epididymal or ejaculatory duct level. Tuberculosis can cause seminal tract obstruction at different levels. Therefore, a history of tuberculosis, especially genitourinary, may be a major clue toward diagnosis of OA. In filarial endemic areas, history of

filariasis could be the cause of obstruction. Similarly, history of schistosomiasis should be asked, especially in endemic areas.

Trauma to inguinal or scrotal areas may lead to OA, especially, if penetrating and is complicated by infection. Surgical history must include history of vasectomy, as well as other inguinal or scrotal surgeries, such as hernia repair or hydrocelectomy, which may raise the possibility of iatrogenic obstruction especially if performed bilaterally.

Presence of other manifestations of cystic fibrosis, such as recurrent respiratory tract infections or family history of similar condition will aid the diagnosis of congenital OA [2, 11, 84].

Low ejaculate volume is often seen in patients with ejaculatory duct obstruction and may be the main presenting symptom together with painful ejaculation or hematospermia [84].

Clinical examination plays a very important role in diagnosis of OA. These patients will have normal virilization with well-developed secondary sexual characteristics. Abdominal examination may show an inguinal scar of prior surgery. Usually a well-trained urologist will be able to diagnose OA on local genital examination. The presence of normal size testis together with fully engorged epididymis will confirm the diagnosis. Epididymal nodule can be palpated on the tail of the epididymis in cases with previous history of STD. Usually, the vas deferens can be easily palpated during examination as a unique cordlike tubular structure. Unilateral or bilateral absence of vas, which may be associated with partial absence of the epididymis, will confirm the diagnosis of congenital vassal aplasia. Other abnormalities on vassal examination may include: beaded vas in case of tuberculosis, thickened vas in case of schistosomiasis, and a vassal defect with or without granuloma in post vasectomy cases [84–87].

Investigations

- **Seminal analysis**—Normal semen volume can be present in cases of epididymal or vassal obstruction but in these cases the fructose in semen is positive. Presence of low semen volume attracts our attention to the possibility of EDO. In these cases, the seminal fluid analysis will also show acidic pH and negative or low fructose due to absence of seminal vesicles secretions [17]. Azoospermia is usually present; however, severe oligoasthenoteratozoospermia may be seen in cases with partial EDO.
- **Hormonal analysis**—These patients will essentially have normal testosterone and gonadotropins levels. Occasionally high normal FSH may be seen on OA patients, which may be due to testicular affection secondary to prolonged obstruction. These cases usually carry poor prognosis following reconstruction surgeries [17, 30].

- **Genetic test**—In cases of bilateral absence of vas, CFTR gene mutation must be done for both the man and woman, especially if they are relatives [17, 85].
- **Radiological**—In cases of OA, radiological diagnosis is used to confirm the diagnosis of EDO and if possible detect the level of obstruction. This can be done through one of the following:
  - **TRUS**—Signs of EDO include presence of midline prostatic cyst, dilated ejaculatory duct or vassal ampulla together with post-obstruction dilation of seminal vesicles (>1.5 cm in width). Presence of prostatic calcifications at the site of ejaculatory ducts can be helpful in diagnosis. It may be difficult to diagnosis cases of fibrotic stricture of ejaculatory duct by TRUS as seminal vesicles may be also affected eliminating the signs of EDO. However, TRUS has low specificity in EDO diagnosis as it is operator-dependent.
    In cases of BAVD, TRUS may show small atrophic or complete absence of one or both seminal vesicles [88, 89].
  - **TRUS-guided seminal vesiculography** is a useful diagnostic tool for EDO. It can confirm the diagnosis in both anatomical as well as dynamic ways. Under TRUS guidance, a 22 G needle is advanced into the seminal vesicle and, after its position is confirmed with aspiration, contrast medium is injected. The presence of sperms in the seminal vesicle aspirate, together with failure of contrast reaching the bladder, will confirm the diagnosis. Sperm found in the aspirate can be cryopreserved for future use in IVF. The major drawback of this procedure is its invasive nature with possibility of injury to pelvic organs and bleeding [90–92].
  - **MRI**—Superior to TRUS in delineating the anatomy of the prostate and distal seminal tract (vassal ampulla, seminal vesicles, and ejaculatory ducts) due to its high soft tissue contrast and multiplanar capability. An MR image serves as a "detailed map" for guiding interventional diagnostic or corrective procedures as it clearly demonstrates the level of obstruction. However, it is expensive and needs a skillful radiologist for proper interpretation of images [93–95].
  - **Abdominal/Pelvic ultrasound** may be needed in cases of unilateral absence of vas deferens to detect ipsilateral absence of kidney [85].

Management of Obstructive Azoospermia

The treatment of OA aims either to achieve pregnancy through intercourse following surgical correction or through ICSI using sperms retrieved from the epididymis or testis.

Counseling

The patients should be properly counseled about both options of treatment. The decision is multifactorial and includes whether the couple has primary or secondary infertility, desired number of children, and history of previous surgery that can affect the success rate of microsurgical reconstruction; e.g., redo-surgery and inguinal obstruction of the vas during hernia repair or vassal or epididymal injury during hydrocelectomy. Proper evaluation of the female partner is also very important in decision-making, especially if her age is above 35 and her chances of achieving normal pregnancy is low, as well as the time needed for surgical reconstruction to function can extend up to 1 year [96]. The financial background of the couple is one of the detrimental factors, as cost-effective analysis has proved reconstruction to be the most effective for a patient with epididymal or post vasectomy obstruction [97]. Risk associated with ICSI such as ovarian hyperstimulation, multiple gestations, and congenital anomalies must be discussed with the couple [98–100].

Surgical Correction of Obstructive Azoospermia

- **Vasal Obstruction**—Vasectomy is one of the most common procedures done for male sterilization and around 10% of these will ask later for reversal. The success rate of microsurgical reconstruction vasovasostomy (VV) reported more than 90% patency with pregnancy rates from 40 to 90%. The time since vasectomy is inversely proportional to a successful surgery [101]. Presence of sperm granuloma, quality of the proximal vassal fluid intraoperatively, length of the proximal vasal stump, and the microsurgical experience are main factors affecting outcomes of microsurgical VV. The techniques utilized for microsurgical VV are two layers anastomosis. However, a few modifications of the standard technique, such as posterior full-thickness sutures, have been added with comparable outcomes to the two layers technique. The patient will be requested to do the seminal fluid analysis in 1 month postoperatively.
- **Epididymal Obstruction**—Microsurgical reconstruction of epididymal obstruction, vasoepididymostomy (VE), success rate has reported 30–70% patency rates and pregnancy rates of 20–50%. The most important predictor of success is the microsurgical experience and the level of epididymal obstruction, with the obstruction at the tail and lower body having the best chances of positive outcomes compared to obstruction at the head with smaller tubules and more difficult anastomosis. The most common technique used now for VE is the end-to-side intussusception technique. Being very delicate anastomosis, the patient will be requested to repeat the seminal fluid analysis in 6 months time and it may take up to 1 year for the anastomosis to function.

Table 8.2 Methods used for sperm retrieval in OA

Source	Procedure
Epididymal	MESA (microsurgical epididymal sperm aspiration) PESA (percutaneous epididymal sperm aspiration)
Testicular	TESA (percutaneous testicular sperm aspiration) TESE (testicular sperm extraction)

In case the couple opted to go for surgical correction it is always advised to have sperms cryopreserved for possible use in the future, if pregnancy was not achieved after successful surgery or failed ones.

- **Ejaculatory duct obstruction**—Surgical correction of ejaculatory duct obstruction is through transurethral resection of ejaculatory ducts (TURED), usually accompanied with vasography as diagnostic and confirmation of the patency following resection of the ejaculatory duct. In up to 75% of cases, sperms will return to the ejaculate and in 25% can achieve normal pregnancy. These patients should be informed that complication rates can reach up to 20% and varies from transient as hematuria, hematospermia, or chronic due to reflux of urine leading to epididymitis with chances of secondary epididymal obstruction or watery ejaculate.

Surgical Sperm Retrieval

Sperm retrieval for OA is done either during surgical correction for possible future use or primarily if the patient opted to go for ICSI. Methods used for sperm retrieval are listed in Table 8.2.

The choice of method depends on the surgical and embryologist preference with no impact on the ICSI outcome [102]. Similarly, using fresh sperms or freezing the sperms for future use has no impact on ICSI outcomes with clinical pregnancy rates of 30–40% and delivery rate of 30% [103–106].

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Chapter 9
Klinefelter Syndrome

Haitham El Bardisi and Ahmad Majzoub

Introduction

Klinefelter syndrome (KS) was first described in 1942 following a report on 9 men with features of hypogonadism and infertility [1]. Discovery of its genetic foundation occurred later in the 1950s when an extra sex chromosome was detected in the genetic profile of this subset of patients [2]. Certainly, KS is the most common chromosomal abnormality, occurring in about 1 in 580 newborn males [3]. It is encountered in 3% of infertile men, overall, and in 11% of azoospermic men [4]. Despite its high prevalence, KS remains profoundly under-detected with only a quarter of patients ever diagnosed during their lifetime, and fewer than 10% diagnosed before puberty [5, 6]. Reasons are mainly derived from the substantial variation in phenotypes and from the discrete nature of symptoms.

The management of children, adolescents, and men with KS has long been a province for endocrinologists with only marginal interest given to the reproductive implication of the disease. However, the advancements witnessed in recent years that resulted in successful sperm recovery, pregnancy, and live births for men with KS inspired reemergence of interest in the study of KS over the past decade.

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Genetic Background

Initial genetic reports determined the presence of a 47XXY karyotype among patients with KS [2]. However, further studies have shown that such a karyotype is in fact present in 80–90% of cases, whereas the remaining 10–20% of cases have a mosaic karyotype (46XY/47XXY), a super-numerous sex chromosome (48XXXY or 48XXYY), or structurally abnormal sex chromosomes [6]. Out of 4477 KS patients reported in a national survey from Denmark and a cohort study from the United Kingdom, 86.3% had the 47XXY karyotype, while the remainder karyotypes are shown in Table 9.1 [6, 7].

The principle genetic cause of KS is meiotic nondisjunction. Nondisjunction is a condition characterized by failure of homologous chromosomes or sister chromatids to properly separate during cell division, resulting in daughter cells with abnormal chromosome number or otherwise known as aneuploidy.

It is generally agreed that most autosomal trisomies result from maternal meiotic nondisjunction, with less than 10% originating from paternal errors [8]. Contrary to this, the condition is different in sex chromosomal aneuploidy such as KS, where a paternal origin for meiotic nondisjunction occurs in almost 50% of cases [8, 9]. When KS results from an extra maternal X-chromosome, it probably occurs due to nondisjunction during the first or second meiotic divisions, whereas when the additional X-chromosome comes from a paternal origin, nondisjunction of the first meiotic division is the only possibility, as a meiosis II error will result in either XX or YY gametes (Figs. 9.1 and 9.2) [8]. Mosaic forms of KS, on the other hand, arise either from nondisjunction in an early mitotic division of a normal 46XY zygote, or from loss of one of the extra X-chromosomes of a 47XXY conception, a process called trisomy rescue. Maternal age is a well-known risk factor for KS. Bojesen et al. detected a fourfold increase in the prevalence of KS cases when maternal age was greater than 40 years [6]. Paternal age, however, has been considered less influential [10]. A recent review by Fonseka et al. confirmed lack of evidence correlating paternal age with autosomal aneuploidy but depicted a minor effect on sex chromosomal trisomies [11]. Other studies did find an influence for advanced paternal age but only in fathers with paternally inherited KS offspring [12].

Table 9.1 Karyotype distribution among 4477 Klinefelter syndrome patients [6, 7]

Karyotype distribution	Klinefelter syndrome patients
47XXY	3863 (86.3%)
46XY/47XXY	383 (8.5%)
48XXXY	157 (3.5%)
49XXXXY	66 (1.5%)
48,XXY + trisomy of chromosome 18	5 (0.1%)
47,XXY/48,XXXY	2 (<0.01%)
Undetermined	1 (0 < 0.01%)

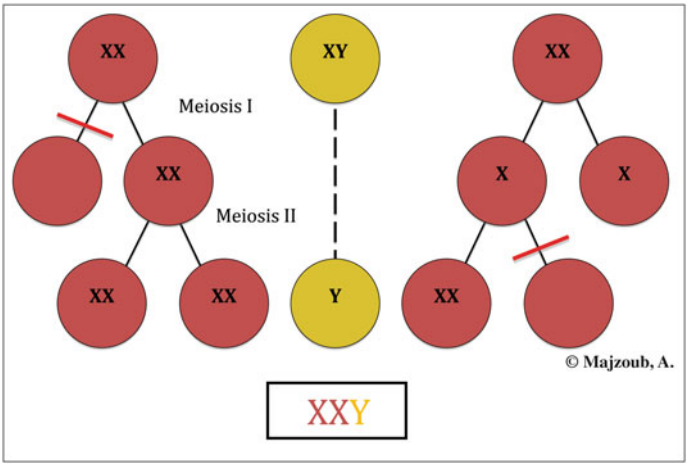
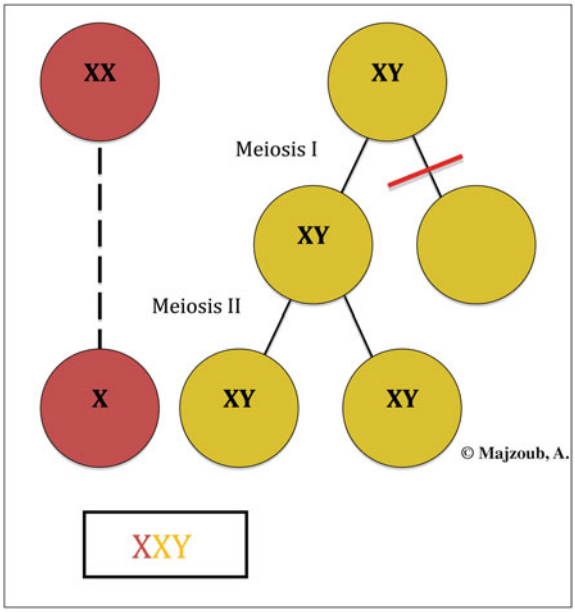


Fig. 9.1 47XXY of maternal origin

Fig. 9.2 47XXY of paternal origin



KS is portrayed through a wide phenotypic spectrum ranging from normal or near normal presentation to poor virilization, sterility, and learning or behavioral problems manifesting at an early age. It is unknown why certain men present on one end of the phenotypic spectrum while others present on the other. Genomic imprinting, a phenomenon where certain genes are expressed while others are silenced in a parent-of-origin-specific manner [9], may help explain this variability.

Another explanation depends on the degree of gene polymorphisms occurring secondary to X-chromosome inactivation. The androgen receptor (AR) gene located on the X-chromosome, and encoding the AR contains a highly polymorphic trinucleotide (CAG) repeat sequence. The length of this CAG repeat is inversely related to the functional response of the AR. In other words, the shorter the AR CAG repeat sequence the more marked the androgen effect is. Patients with KS appear to have an inactivation of the shortest AR CAG repeat in a process called skewed or nonrandom X-chromosome inactivation [13]. Patients having short AR CAG repeat lengths generally respond well to androgen therapy and are able to form more stable partnerships and achieve a higher level of education compared with long CAG repeats. On the contrary, patients with long CAG repeat lengths generally have an increased body height and arm span, decreased bone density, decreased testicular volume, and gynecomastia [13]. This nonrandom X-chromosome inactivation might also explain the diversity of the KS phenotype.

Endocrine Function and Spermatogenesis in Klinefelter Syndrome

There is evidence suggesting that infants with KS have an initial embryonal testicular development that is similar to 46XY infants. Germ cells originating in the yolk sac undergo migration to the urogenital ridge [14]. This is followed by the initiation of secretory function of mesenchymal (nacent Leydig) cells and Sertoli cells [14]. Hunt et al. demonstrated a normal number of migrating germ cells to the genital ridge followed by a reduction in their mitotic proliferation and expansion as the testis develops [15]. The latter was confirmed by Mikamo et al. who displayed a progressive diminution in the number of spermatogonia from 24 to 0.1% of control value, over the course of the first year of life [16]. Studies performed on the prepubertal KS boys reveal marked reduction in the number of spermatogonia with evidence of differentiation arrest at the spermatogonium or early primary spermatocyte stage [17]. Moreover, immature Sertoli cells also have reduced capability of transforming into adult mature cells [18].

This raises an argument on how spermatogenesis in KS patients occurs. Two hypotheses have been brought forward. The first one is based on the notion that some 47XXY spermatogonia are capable of completing meiosis, which would explain the increase in sex chromosomal aneuploidy rate present [17, 19]. The second hypothesis suggests that KS patients may have patches of 46XY spermatogonial stem cells and the higher aneuploid sperm results from meiotic errors caused by a compromised testicular environment [20]. Certainly, testicular histopathologic evaluation of adult KS patients demonstrates patchy areas of extensive fibrosis and hyalinization of seminiferous tubules [21] interspersed with areas containing larger more differentiated cellular structure [22]. Intuitively,

however, one would argue against the latter hypothesis, especially because the majority of KS patients are nonmosaic.

Contrary to sperm production, endocrine function in KS patients appears to follow a normal pattern until puberty. Several studies have indicated that prepubertal KS boys have normal serum levels of testosterone, follicular stimulating hormone (FSH), luteinizing hormone (LH), and inhibin B until the onset of puberty [23–25]. After an initial rise in serum testosterone, the level plateaus remains consequently at a low–normal range throughout puberty [24, 25]. Such level appears to be sufficient for KS boys to progress through puberty and develop satisfactory secondary sexual characteristics [23, 26].

Serum estradiol levels are generally high among KS prepubertal and pubertal boys, irrespective of the presence or absence of gynecomastia, with a tendency to have higher estradiol/testosterone ratio throughout puberty [23, 27]. Patients with KS exhibit an initial increase in inhibin B similar to normal boys before the clinical onset of puberty, but the levels soon drop thereafter in association with a simultaneous increase in serum testosterone [18, 24]. From mid-puberty onward, a gradual increase in FSH and LH concentrations is seen in KS patients, with FSH levels increasing somewhat earlier and more markedly than do LH levels [23, 24, 27].

Clinical Manifestations

Case Scenario 1

A 14-year-old boy presents with a delay in puberty. He has a history of learning difficulty since 8 years of age characterized by troubles with spelling and composition. Although he required additional help at school, his IQ was normal and he was able to progress academically along with his peers. An impulsive behavior was noted during his psychological evaluation, however without sufficient findings to support a diagnosis of attention deficit disorder. His physical examination reveals tall stature (99 percentile for age), gynecomastia, and small testes. A chromosomal study reveals a 47,XXY karyotype.

Case Scenario 2

A young adult man presents with primary infertility of 2 years duration. He has no significant past medical history apart from a slight delay in puberty. He reports normal sexual activity with good libido, normal erection and ejaculation. His physical examination reveals no gynecomastia, normal penile size, and bilateral testicular atrophy. A chromosomal study reveals a 47,XXY karyotype.

The two case scenarios signify the variability of the clinical presentation of patients with KS according to their age and degree of phenotypic affection. Before puberty, it is usually difficult to recognize boys with KS for the principle reasons that were described in the previous section. Discrete physical anomalies such as a mild decrease of testicular volume and increase in lower limb length may be seldom noticed. After puberty, small firm testes and varying symptoms of androgen deficiency characterize the syndrome. These symptoms can be classified into the following:

- *Consequences of Hypogonadism*: a delay in the development of normal secondary sexual characteristics is typically present. Reduced facial and body hair has been reported by about 20% of KS patients [28]. While most patients achieve normal penis size, approximately 70% of patients complain of falling libido and potency from the age of 25 years [29].
- *Gynecomastia*: KS patients have a 50% higher incidence of gynecomastia [30], attributed to testosterone deficiency and/or reduction in testosterone/estradiol ratio [31].
- *Anthropometry and Body Composition*: an accelerated growth is usually seen among KS patients from childhood resulting in a taller stature than their normal counterparts [30]. Although KS patients generally have a normal body mass index (BMI), they typically have an increase in body fat and a decrease in lean muscle mass resulting in an unfavorable muscle/fat ratio [32]. Our understanding of the androgenic effect on body fat mass is centered on investigations on hypogonadal aging men. Androgens have a negative effect on adipose tissue development as they inhibit the differentiation of pluripotent cells into fat cells [33]. Nonetheless, the increase in body fat in KS patients is witnessed even before puberty, suggesting a possible genetic influence on this observation [34].
- *Bone Mineralization*: Hypogonadism is a known cause of secondary osteoporosis in both men and women [35]. Many studies conducted on KS patients revealed a significant reduction of bone mineral density [36, 37], and a higher incidence of osteoporotic fractures of the hip, spine, or forearm [7, 38]. Such fractures were responsible for higher hospital admissions, employing significant morbidity and mortality on KS patients [7].
- *Diabetes and Metabolic Syndrome*: An association between diabetes mellitus and KS has been established for reasons that remain poorly understood. The reported incidence has been between 15 and 50% [39] and is mainly attributed to genetic factors, endocrine imbalance, or autoimmune mechanisms. In a study of 70 KS patients who were compared to an age-matched control group, Bojesen et al. revealed a significant incidence of metabolic syndrome and insulin resistance [40]. Plasma low-density lipoprotein (LDL) cholesterol was increased while high-density lipoprotein (HDL) cholesterol was decreased compared with the control group [40].
- Hypogonadism is an established risk factor for metabolic syndrome and diabetes mellitus. However, this relationship appears to be secondary to adiposity rather than testosterone itself. Testosterone replacement was successful in improving

insulin sensitivity only in obese and not in lean hypogonadal men [41]. It is not known whether this is the governing rule in KS patients or if there may be other genetic influences that are responsible. Jiang-Feng et al. detected a higher prevalence of diabetes mellitus among KS patients compared with a group of idiopathic hypogonadotropic hypogonadism patients [42]. Their report also revealed that testosterone deficiency was not the only impelling factor for this finding, suggesting a potential genetic influence especially because the incidence of diabetes mellitus was significantly higher among mosaic (46XY/47XXY) compared to nonmosiac 47XXY patients in the study population ($p = 0.008$).

- *Cardiovascular Disease*: Mitral valve prolapse has been found to commonly affect KS patients, placing them at a greater mortality risk [43]. Clotting defects with subsequent embolic events were also identified. One study reported an increased activity of plasminogen activator inhibitor-1 responsible for dysfunctional fibrinolysis [44]. In another recent study, an increased platelet activity has been detected [45]. High levels of C-reactive protein, LDL cholesterol, and triglycerides and low levels of HDL cholesterol were proposed as factors placing patients with KS at risk for ischemic heart disease. Adiponectin, an adipocyte-specific secretory protein having anti-atherogenic, anti-inflammatory, and anti-diabetic properties [46], has been found to be inversely proportional to testosterone level; a fact that helps explain the cardiovascular adverse effects of testosterone replacement [47]. Remarkably, patients with KS were found to have higher levels of adiponectin irrespective of their testosterone level [32]. This suggests that despite the previously mentioned risk factors, KS patients are in fact safeguarded against ischemic heart disease. Epidemiological data on mortality in patients with KS have found an increased mortality from diabetes, but the mortality from ischemic heart disease was significantly decreased [7].
- *Cancer*: A 50-fold increased risk of breast cancer has been detected in KS patients [48]. Mediastinal germ-cell tumors have also been reported in more than 40 patients [49], most of them developing before the age of 30 years. Moreover, hematologic malignancies, such as leukemia and lymphoma (non-Hodgkin), have also been linked with KS [50]. Conversely, a recent study revealed a significantly decreased risk of death from prostate cancer [51], which expectedly is secondary to hypogonadism.
- *Cognitive Disturbances*: Deficits in very specific domains of cognition such as language and executive functions are commonly associated with the syndrome. Activities, such as concept formation, problem solving, task switching, speed of response, and planning, which seem similar to those observed in cytogenetically normal dyslexic children, are particularly affected [52].
- *Psychiatric Disturbances*: Studies from the late 1960s that were performed on prison inmates and psychiatric hospitals revealed an increased incidence of psychiatric illness among individuals with KS [53]. Ratcliffe et al. tracked patients with KS for a long duration and reported an increased frequency of referrals for psychiatric treatment [26]. A four- to fivefold increased incidence of

KS has been detected in a survey searching for sex chromosome aberrations among patients with schizophrenia. The proposed reason for this association is possibly an overexpression of X-linked genes that escaped the X-chromosome inactivation seen in patients with KS [54].

Diagnosis

The diagnosis of KS requires some degree of clinical suspicion in addition to a high level of awareness about its prevalence in men with delayed puberty, sexual dysfunction, low testosterone, and infertility. The condition can be suspected during the initial male fertility evaluation when signs and symptoms of hypogonadism are evident. Physical inspection usually reveals decreased facial and body hair together with gynecomastia and female fat distribution. Small testes having firm consistency are typically discovered on genital examination. A digital rectal examination may reveal atrophy of the prostate, which was documented in about 30% of cases in one study [55].

Azoospermia on semen analysis is the norm, though not the rule as about 8.3% of patients are able to produce sperm during ejaculation [56]. Endocrine evaluation demonstrates low serum testosterone in about 80% of adult patients with KS [31], secondary to some degree of Leydig cell dysfunction. Serum concentrations of sex hormone-binding globulin (SHBG) are high, resulting in further reduction of free testosterone. Estradiol, on the other hand, is commonly higher in KS patients than in normal men, disrupting the testosterone/estradiol ratio. Concentrations of LH and FSH are elevated, highlighting a hypergonadotropic hypogonadism picture. FSH is most consistent representing coherent damage of seminiferous tubules [57], which in its turn is associated with a decrease in inhibin B level [24].

Early diagnosis and treatment of KS boys should have favorable effects on their physical, academic, and social development and health [58]. Unfortunately, only 10% of men affected by KS are diagnosed in at a young age—the time when treatment may be most effective. Screening for KS in target populations such as children with learning disabilities or developmental problems has been proposed as a method that could offer early detection and treatment [58].

Buccal epithelial testing for Barr bodies, which correspond to the inactive supernumerary X-chromosome, is no longer used [59]. The diagnosis is currently confirmed cytogenetically through chromosome analysis of lymphocytes [59]. Sometimes a normal male karyotype may be reported requiring the use of skin fibroblasts or testicular biopsy samples to confirm chromosome mosaicism. Y chromosome microdeletion analysis is also indicated in KS patients as few reports have suggested an increased risk for such deletions in this population [60].

Because of the decreased level of testosterone and significantly increased risk of osteopenia and osteoporosis in men with low testosterone, bone density testing should be routinely performed in patients with KS. If osteopenia or osteoporosis is

diagnosed, then additional laboratory tests including calcium, phosphorus, parathyroid hormone (PTH) calcium, and vitamin D3 should be measured.

The patients should be made aware of the increased risk of breast cancer and should be taught to perform regular self-breast examinations and seek advice if they detect a change in consistency of the breast tissue or in case there was discharge from the nipples.

Management

A multidisciplinary approach to patient management is vital to confront all features of the disease. Although achieving fertility is a principle goal, the clinician should not undervalue other consequences that may have an impact on patients' quality of life and overall health. A number of factors must be considered such as the age at which the diagnosis was made, the presenting complaint, and whether fertility is pursued.

Managing Hypogonadism

In Adolescents and Adults

The marked hypogonadism experienced by KS boys preventing their progress through puberty needs to be addressed at some point with testosterone replacement therapy (TRT). This is aimed at promoting the development of secondary sexual characters and stimulating linear bone growth and muscle bulk. Evidence present today makes it unknown whether TRT carries a significant impact on fertility in men with KS. In the case series by Schiff et al. [61], five patients received TRT for a duration ranging from 2 to 14 years and in only one patient sperm could be retrieved through testicular sperm extraction (TESE). Furthermore, Ramasamy et al. documented a worse sperm retrieval rate (SRR) among patients with prior exposure to TRT [62]. Current best practice statements recommend initiating therapy in early to mid-puberty, or at the onset of hypogonadism [63–65], to ensure normal completion of puberty and prevent unfavorable effects of long-term androgen deficiency. Other physicians may choose to initiate therapy at an even earlier age aiming to correct decreased penile length, which may be relatively smaller in KS boys, though not to the extent of micropenis [66]. There are no specific TRTs or protocols intended for patients with KS. Age-appropriate formulations and/or doses may be determined according to clinical practice guidelines on the treatment of hypogonadal men [67].

Few reports have looked into hormone replacement therapies that can minimize the deleterious effect of exogenous testosterone or even enhance sperm production. Mehta et al. used topical testosterone in combination with an aromatase inhibitor in

ten patients for a period of 1–5 years before microsurgical testicular sperm extraction (microTESE) and were able to retrieve sperm from seven patients [68]. Other available options include the co-administration of intramuscular injections of human chorionic gonadotropin or clomiphene citrate. Such regimens were not evaluated in KS patients specifically, however, some evidence can be withdrawn from their use in hypogonadal men. In a retrospective analysis of 26 men treated with testosterone replacement therapy along with human chorionic gonadotropin (HCG), there were no differences in semen parameters after one year of treatment [69]. Remarkably, spontaneous pregnancy occurred in 35% of patients. Lower doses of HCG were also investigated and were successful in maintaining adequate intratesticular testosterone levels [70]. One disadvantage for continued HCG use is that FSH replacement should be considered after a period of time to maintain spermatogenesis. Clomiphene citrate also has been acknowledged as a reasonable alternative to exogenous testosterone in hypogonadal men. Despite a lower efficacy than injectable testosterone, clomiphene citrate is capable of achieving similar symptom relief [71]. Enclomiphene citrate, a newer estrogen receptor modulator, successfully normalized endogenous testosterone production and restored sperm counts through reestablishing the function of the hypothalamic-pituitary-testicular axis [72]. Concerns regarding safety of long-term use of clomiphene-like products emerged. Moskovic et al. looked into this issue through a follow-up of 46 patients receiving clomiphene citrate for a period of more than 12 months. No adverse events were detected in the whole group, concluding that it is an effective long-term therapy in appropriate patients [73]. Further studies of larger sample size and longer duration of follow-up are still required to estimate, with great certainty, treatment safety.

Fertility Management

Peripubertal KS Boys

Based on the knowledge, there is a constant decline in testicular function beginning at puberty. Some researchers have proposed the notion to seek fertility preservation at an earlier age even before staring TRT. Indeed, Mehta et al. reported the presence of sperm in the ejaculates of 70% of adolescents with KS aged 12–20 years [74]. Studies on testicular samples are understandably scarce in this age group. In a case report by Damani et al., sperm was successfully retrieved from the testicular tissue of a 15-year-old [75]. In another observational retrospective study, Aksglaede et al. [76] determined an age limit of 10 years after which there would be loss of germ cells from testicular tissue. After examining testicular tissue samples from 14 KS boys aged 10–14 years, Wikstrom et al. [18] were able to find germ cells in only half of them, suggesting the presence of testicular dysfunction in the peripubertal period. Moreover, all samples containing germ cells were from boys younger than

12 years of age with prepubertal-sized testicular volumes, normal serum inhibin B, and FSH concentrations. As such, researchers have looked into markers that can be examined in order to successfully time sperm retrieval. Serum inhibin B levels being normal in the prepubertal and early pubertal periods [24] make it an ideal marker to reflect the integrity and number of Sertoli cells. Unfortunately, this was not the case as seen in a study of Wikstrom et al. [17], where normal inhibin B levels were inconsistent with the presence of spermatogonia. Anti-Müllerian hormone is another marker that failed to denote spermatogenetic activity, although it is known to rise during prepuberty and early puberty in boys with KS and decline afterward [76]. Finally, insulin-like factor 3 (INSL3), a marker of Leydig cell function found to be normal before puberty in KS boys and known to decrease thereafter [77], does not correlate with testicular spermatogenic function.

Cryopreservation in Adolescents

Ethical concerns and challenges arise when obtaining sperm via masturbation from adolescent patients. Anejaculation or idiosyncratic masturbation that is common in this age group would further aggravate the challenge. The clinician should be able to approach this sensitive issue in an age-appropriate way and actively involve parents in a productive manner. Cryopreservation of sperm is currently offered to adolescents after a thorough discussion with them and their parents. Current recommendations are to bank sperm for all males at or above Tanner stage III [78], especially if viable sperm are found on semen analysis.

If masturbation cannot be accomplished, some have advocated the use of stimulatory techniques. Vibratory stimulation or, alternatively, electroejaculation under anesthesia can be attempted. Data on the use of these procedures are mainly drawn from studies performed on fertility preservation in cancer patients. In a study of patient and parent attitudes toward sperm preservation in boys undergoing chemotherapy, 70% were in favor of using masturbation or electrostimulation as a means of obtaining sperm for cryopreservation [79]. Invasive procedures to retrieve mature sperm from adolescents are currently not justified because of their negative endocrine effects and the high retrieval rates seen in adulthood.

A newer modality for fertility preservation has been proposed where testicular tissue cryopreservation is offered in the hope of preserving spermatogonial stem cells (SSCs). These cells would later be used to restore spermatogenesis, or they could be matured in vitro to produce viable sperm [80]. The experimental nature of the whole procedure, combined with the need for a surgical procedure on minors, does not justify its use as an alternative option at the moment. Especially that germ cells may not be identified in up to 50% of pre-adolescent boys undergoing testicular tissue biopsy [18]. Further research should focus on the feasibility of this process as well as on factors that can help identify potential subjects who would most likely benefit from testicular tissue freezing.

Adult Klinefelter Syndrome Men

During the past 15 years, treatment of the infertility associated with KS witnessed major advancements that made it possible for patients today to father their biologic children. Although spontaneous pregnancy has been reported in extremely rare occasions, intracytoplasmic sperm injection (ICSI) is perhaps the more realistic approach through which pregnancy is achieved. Few cases of successful pregnancies have been reported after ICSI with ejaculated sperm from KS patients [81]. Despite that, the majority of patients present with azoospermia and would require a sperm retrieval procedure before ICSI. Testicular sperm extraction (TESE) is a well-established procedure that has been utilized for sperm retrieval in patients with nonobstructive azoospermia for exactly 2 decades. Its use in KS patients also yielded encouraging results with reported SRR ranging from 30 to 70% [68, 82]. A higher success rate is generally achieved when microsurgical TESE is utilized as shown in recent comparative reviews [83]. It entails the use of a surgical microscope where up to 25× magnification can be used to allow more precise visualization and sampling of dilated sperm-containing tubules. Almost 50% of all published cases of KS patients in whom TESE and ICSI was performed achieved pregnancy and live birth [83]. In a recent study, we evaluated the clinical outcomes of 43 nonmosaic KS patients who underwent ICSI following TESE. A statistically significant higher sperm retrieval rate was detected in patients undergoing microTESE in comparison with conventional TESE. Moreover, patients receiving presurgery hormonal treatment, specifically with an aromatase inhibitor, did significantly better than those who underwent surgery without hormone therapy [82].

Hypogonadal patients scheduled for TESE should be treated preoperatively to preferably normalize their serum testosterone level. Aromatase inhibitors, human chorionic gonadotropin, and clomiphene citrate have all been tried in KS patients [61]. Based on disease characteristics, such as an increased fat mass and a disrupted testosterone/estradiol ratio, some have proposed that an aromatase inhibitor would be superior to other therapies [82]. Such medications have been found to increase testosterone-to-estradiol ratio, sperm concentration, and motility in controlled studies [84]. However, they should be used for no more than 2 months before surgery as tachyphylaxis would develop.

Several reports have looked into predictors of success with TESE. Baseline testosterone level and response to medical therapy are generally favorable, although not supported in some studies. Levels of FSH and inhibin B, which have been considered predictive for adequate spermatogenesis in men with normal karyotype [85], do not seem to carry the same significance in patients with KS. In fact, even patients with inhibin B below the detection limit underwent successful TESE in 1 study [86]. Others have suggested that the patients' age at TESE might be of significance based on the progressive deterioration of testicular function that is established in KS patients [87]. A factor that was not consistent in other studies [82, 88]. Furthermore, no association between outcome of TESE and testicular volume, preoperative ultrasonography results, or degree of virilization has been found [88].

Genetics Risks to Offspring

With the current witnessed advancements and the hope KS patients have in fathering children, the concern of passing some form of genetic abnormality to their offspring rises. In a questionnaire-based survey of patients with KS, although the majority of respondents did express a desire to father children [89], 70% of them admitted to having safety concerns in transmitting chromosomal or developmental abnormalities to their offspring [89]. Investigations of ejaculated or testicular sperm from KS patients reveal a high level of abnormal sperm morphology [90]. Therefore, it has been proposed that patients with KS have a substantially higher proportion of aneuploidy in their sperm in comparison with normal males [58]. Conversely, the majority of offspring born to men with KS have been healthy, with a normal chromosomal karyotype [91], suggesting that normal haploid sperm may only be able to fertilize an ovum and cause pregnancy. Nonetheless, conception of 47,XXY pregnancies has been reported [92].

Given the likelihood of higher chromosomal abnormalities in the offspring of men with KS, preimplantation genetic diagnosis (PGD) of embryos has been recommended. Staessen et al. [93] have argued that PGD is a requirement, as sometimes the morphology of the embryo does not preclude underlying genetic abnormalities. They confirmed, in their series, that KS patients had a lower rate of normal embryos than normal controls (54% vs. 77.2%), and that the abnormalities can occur on sex chromosomes as well as autosomes. On the other hand, PGD may not be available in most centers due to its added expense or possible expertise requirement. Not to mention that it is disfavored by a number of religious and/or cultural groups. Bottom line, PGD in KS couples undergoing ICSI should be considered, provided there is no objection to its use.

Conclusion

KS is a common chromosomal abnormality imposing significant effects on patients' overall health and fertility. It is characterized by hypergonadotropic hypogonadism and testicular failure, which appears to be progressive in nature with relatively better testicular function witnessed among younger boys. Early treatment of adolescents is thus recommended to overcome the negative influences of hypogonadism. Adolescents interested in fertility should be asked to provide a semen sample and should be offered sperm cryopreservation. However, invasive procedures are impractical and costly and can have negative consequences, especially on hormone levels. Adult sperm retrieval is promising with reported success rates as high as 70%. Pretreatment medical therapy delivers superior results with a potential advantage to anastrozole. Couples concerned about the transmission of chromosomal abnormality to their offspring may be offered PGD during ICSI.

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Chapter 10
CFTR-Related Male Infertility

Victoria McKay and Alan Fryer

Clinical Vignette

Simon Jones (31 years old) and Heather Simpson (29 years old) are a healthy, unrelated Caucasian couple who have been trying for a pregnancy without success for the last 16 months. On investigation, Simon has follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels and testis volumes all within the normal range. His ejaculate is found to be azoospermic, and he is diagnosed with obstructive azoospermia (OA). Clinically, the vas deferens is not palpable on each side. Trans-scrotal ultrasound confirms congenital bilateral absence of vas deferens (CBAVD). After counseling, Simon proceeds to have a sample of peripheral lymphocyte blood analyzed for 50 common mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. He is found to carry 2 *CFTR* mutations: a common mutation associated with classical cystic fibrosis (CF), known as p.Phe508del, and a mutation associated with mild *CFTR*-related disorders (*CFTR*-RD), known as c.350G>A (previously known as R117H or Arg117His). Neither Simon nor Heather has any family history of CF (Fig. 10.1) or past medical history of respiratory, pancreatic, or nasopharyngeal symptoms.

Heather proceeds to have DNA analysis for the same 50 common *CFTR* mutations, and no mutations are found. The couple are reassured that their risk of having a child with classical CF is low, and Simon undergoes successful micro-surgical epididymal sperm aspiration (MESA). In vitro fertilization (IVF) with

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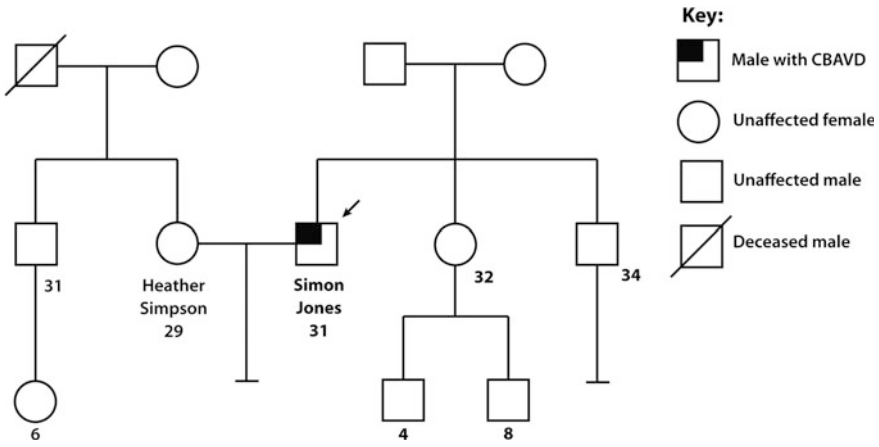

Fig. 10.1 Pedigree for Simon Jones and Heather Simpson

intra-cytoplasmic spermatozoa injection (ICSI) is performed, and the couple progress to a clinical and biochemical pregnancy. Simon's parents and siblings are advised to seek CF carrier testing via their general practitioners and the couple go on to have a healthy male child born at term. The baby boy undergoes neonatal heel prick screening for conditions including CF, which reveals normal immunoreactive trypsinogen (IRT) levels. Heather and Simon are advised to inform their son that he may wish to access genetic carrier testing for CF before starting his own family in the future.

What Is *CFTR?*

Cystic fibrosis transmembrane conductance regulator (CFTR) is the protein product of the *CFTR* gene that was first isolated in 1989, after a long international research effort to find the causative gene for the human genetic disorder cystic fibrosis [1–3]. The *CFTR* gene is located on chromosome 7 at band 7q31.2, and Riordan et al. identified 24 exons in the *CFTR* gene [2]. The sections of DNA within a gene that will encode the final protein molecule (in this case CFTR) reside within "exons" and the intervening DNA segments are termed "introns." During transcription of the DNA sequence into RNA, all the exons and introns are transcribed but then the RNA molecule is modified, removing the intron sequences via a process known as "splicing." The molecule also has other modifications made to it (a methyl cap—a methylated guanosine molecule—is added to the 5′ end and a polyA tail to the 3′ end of the gene) before a mature messenger RNA (mRNA) molecule is produced. Pathogenic mutations are usually found within the exons of a gene or at the

exon-intron boundaries. These boundaries are the sites at which splicing occurs and genetic variation at these points could result in either the failure to insert exon sequences (known as "exon skipping") or incorrect insertion of intronic sequences into the final mRNA molecule.

The CFTR protein functions as a cyclic adenosine monophosphate (cAMP)-regulated chloride- and bicarbonate-conducting channel [4]. *CFTR* mutations cause abnormal flow through this channel and lead to defective fluid and electrolyte transport. This mechanism is thought to be a major contributor to the respiratory, pancreatic, and nasopharyngeal symptoms in CF [5].

Every person carries 2 copies of the *CFTR* gene, and common nomenclature refers to any mutations on the same chromosome (or allele) as in *cis* and mutations on opposite alleles as in *trans*. Any person with a *CFTR*-related phenotype will have pathogenic mutations in both copies of their *CFTR* gene (i.e., the mutations will be in *trans*) and will have inherited one mutation from each parent (that parent being a "heterozygote" or healthy carrier). Sometimes, the mutation will be the same in both alleles, and under these circumstances, the patient is described as being a "homozygote" for the mutation. In other cases, the 2 mutations are different and the patient is then described as a "compound heterozygote."

Mutations in the *CFTR* gene can result in a spectrum of clinical phenotypes, which can include CF, CBAVD, bronchiectasis, idiopathic pancreatitis, and sweat chloride elevation without CF. A simple subdivision would be into classical CF on the one hand and the milder *CFTR*-related disorders (*CFTR*-RD) on the other.

CF is the most common lethal genetic disorder in Caucasians, with an incidence reported at between one in 3500 [6] and one in 2500 [7, 8], giving an estimated carrier frequency of one in 25 [9]. Patients with classical CF manifest multi-organ dysfunction, chronic lung infection and inflammation, pancreatic insufficiency and infertility. *CFTR*-RD include patients with biallelic mutations (i.e., mutations in both alleles) of the *CFTR* gene who present with milder, atypical and late-presenting forms of CF—including isolated CBAVD or CBAVD with mild or atypical CF features, such as CBAVD with bronchiectasis.

How Common Is *CFTR*-Related Male Infertility?

Infertility is seen in around 15% of couples worldwide, and 40% of cases have a male cause [10]. Azoospermia (the absence of spermatozoa in the ejaculate) can be classified as non-obstructive or obstructive, and CBAVD accounts for 6% of cases of obstructive azoospermia (OA) and 1–2% of all cases of male infertility [11]. In male infertility secondary to OA, cases diagnosed with CBAVD using strict criteria are commonly caused by pathogenic *CFTR* mutations [12, 13]. Indeed, Dequeker et al. suggest that 80% of patients with isolated CBAVD have 2 identifiable pathogenic *CFTR* mutations, usually in a compound heterozygous state [12].

How Does *CFTR*-Related Male Infertility Present?

Male patients with *CFTR*-related infertility may present in a variety of ways: a patient with classical CF diagnosed in childhood who is referred for fertility discussions when transitioning to adult services; a male patient diagnosed with mild or atypical CF in early adulthood who is found to be azoospermic; a patient with azoospermia who has no palpable vas deferens on examination; or a patient who is diagnosed with OA secondary to CBAVD found on imaging. The latter examples of a male patients with OA secondary to CBAVD who are subsequently shown to have 2 mutations in CBAVD are the most common presentation of *CFTR*-related male infertility.

How Do *CFTR* Mutations Cause Male Infertility?

Of men with biallelic *CFTR* mutations, 95–97% are infertile due to a combination of viscous semen and absence of Wolffian duct structures, commonly presenting as CBAVD [14]. A fetal anomaly in the seventh week of gestation results in the characteristic anatomic pathology of CBAVD, including absent vasa and complete or partial absence of epididymis [15]. No female equivalent of CBAVD exists, due to the different embryological origin of the female reproductive tract.

Not all cases of CBAVD are caused by *CFTR* mutations, but in males with either unilateral or bilateral congenital absence of vas deferens (CAVD), *CFTR* mutation testing is indicated [16, 17]. Diagnostic criteria for *CFTR*-related CBAVD have been outlined (Table 10.1) [12] and could be used as a prompt to initiate *CFTR* testing in males with these clinical features.

CFTR-related male infertility might thus be suspected based on a combination of clinical features: demonstration of OA with typical biochemical and clinical

Table 10.1 Diagnostic criteria for *CFTR*-related CBAVD

Clinical feature	Further definition or laboratory values
Azoospermia	
Low seminal fluid volume	<2.0 ml
Typical biochemical features	pH < 7.2, absent/decreased fructose and α(alpha)1–4 glucosidase
Absence of palpable vas deferens	Absent on trans-scrotal USS
Anatomical abnormalities on *trans*-rectal ultrasound	Absence of intra-abdominal tract of vas deferens, globus major, and hypoplasia of the seminal vesicles
FSH, LH, and testosterone levels within normal limits	See local laboratory limits

Adapted from [12]
USS ultrasound scan, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone

findings of absent vas deferens on clinical examination or trans-scrotal ultrasound (Table 10.1). The finding of 2 CF mutations on the molecular testing of peripheral blood lymphocytes, often in compound heterozygous state, in a male patient with CBAVD confirms *CFTR*-related male infertility. Depending on the genotype, further clinical evaluation for features of classical or atypical CF may be required.

Can *CFTR* Mutations Cause Non-CBAVD Infertility?

The question of whether *CFTR* mutations can cause non-CBAVD infertility was raised by Van der Ven et al., who screened 127 unrelated, healthy males attending infertility clinics for 13 common *CFTR* mutations [18]. They found a single *CFTR* mutation in 17.5% of the men attending with reduced sperm quality and in 9.5% of men attending with azoospermia. No *CFTR* mutations were found in the 26 men with normozoospermia. The authors reported an increased frequency of mutations, including the IVS8-5T allele, in non-CBAVD azoospermic males compared to both fertile males and the general population incidence. The conclusion of this study was that heterozygous mutations in *CFTR* may confer a risk of non-CBAVD male infertility [18].

Pallares-Ruiz et al., however, compared the *CFTR* genotypes of men with severe oligoasthenoteratozoospermia (OAT) to matched background controls and found no significant differences in the frequency of *CFTR* mutations [19]. The authors concluded that neither the IVS8-5T allele (see later) nor any other *CFTR* mutations were implicated in OAT and that the findings by van der Ven et al. may have been due to undetected CBAVD cases. Further studies have also supported this theory [20]. There is therefore no firm evidence that heterozygosity for *CFTR* mutations, the IVS8-5T variant included, confers an increased risk of non-CBAVD male infertility.

Does the Type of *CFTR* Mutation Determine the Phenotype?

More than 2000 *CFTR* mutations have been described to date [12, 21–23], and they are often individually rare, making genotype–phenotype correlations complex, especially as clear phenotypic categories are difficult to define [24]. The frequencies of specific mutations vary depending on geographical and ethnic backgrounds [9, 25]. The most common *CFTR* mutation is p.Phe508del in exon 11, accounting for 75% of all UK mutations [26]. The p.Phe508del mutation has historically been described in various ways, including Phe508del and Δ(Delta)F508, but the most accurate current nomenclature is c.1521_1523delCTT [23]. This type of mutation nomenclature gives the exact changes of the base pairs and describes deletion of the

nucleotides CTT between positions 1521 and 1523 of the *CFTR* gene. For ease of recognition, however, the protein coding nomenclature, p.Phe508del, for this mutation will be used throughout the chapter. All other mutations are described using their current coding DNA (cDNA) names.

Classification of *CFTR* mutations based on their effect on *CFTR* protein function and production is widely quoted; however, many *CFTR* mutations have a number of different functional consequences [8, 12, 27]. Nevertheless, some mutations are more likely to confer a phenotype of classical CF, whilst others might be associated with no clinical features, isolated CBAVD or CBAVD with mild or atypical CF features, such as CBAVD with bronchiectasis.

How Are *CFTR* Mutations Detected and Reported?

Guidelines were produced in 2006 based on expert consensus opinion, to recommend a standard practice for testing and reporting of mutations in *CFTR* [12]. Both targeted (level 1, see later) and scanning methods (level 2, see later) exist. The former techniques detect known mutations that are common in the local population or test for a previously identified familial mutation; the latter involves more extensive sequencing of the whole *CFTR* gene. No single method for mutation detection is recommended, but laboratories can advise on the limitations of testing via their chosen method. Laboratories will make efforts to detect all mutations occurring at a frequency of >1% in the local population; therefore, if a patient is of an ethnic origin not commonly seen within the local population, further discussion with the local laboratory will be needed. In Liverpool and most of the UK, the standard practice is to test for the 50 most common CF mutations that occur in the local population using a commercial kit; the 50 mutations include p.Phe508del, and the next most common mutations c.1624G>T (previously known as G542X), c.1652G>A (G551D), c.3846G>A (W1282X), and c.3909C>G (N1303K); each of which accounts for only 1–2% of known mutations.

Many causative mutations remain undetected in patients with atypical presentations of CF, including CBAVD. They seem to be harder to detect in non-Northern European populations. Reasons for this are unclear but perhaps due to lack of commercial kits for targeting mutations in non-Northern European populations. Undetected *CFTR* mutations may lie in introns or regulatory regions that are not routinely analyzed by laboratories. Large *CFTR* gene rearrangements occur in 1% of CBAVD patients and can also escape detection using conventional amplification assays [12].

Searching for unknown mutations by extensive sequencing may detect sequence variants of uncertain significance. The terms “mutation” and “sequence variant” are synonymous and do not denote functional consequences or disease association [12]. The term “mutation” has been used throughout this chapter for the purposes of consistency. Similarly, the term “polymorphism” refers to a mutation with an allelic

frequency of greater than 1% in the general population and does not mean that the mutation has no clinical consequences. A number of criteria are used to determine whether a sequence variant is likely to be causative (i.e., a pathogenic mutation). Such considerations include the following: (1) whether the mutation results in an amino acid change that severely affects *CFTR* synthesis or function; (2) the introduction of a premature termination signal (leading to shortening of the *CFTR* protein) and (3) variants that alter invariant intron splice sites and delete one or more exons of the *CFTR* gene [12]. If a mutation has been reported previously in patients with CF or *CFTR*-RD, laboratories can be more confident in classifying it as likely to be pathogenic. However, functional studies have only been undertaken on a very small number of mutations, meaning data about their effect on *CFTR* protein structure or function are lacking.

Which *CFTR* Mutations Are Common in CBAVD Patients?

In a large systematic review, Yu et al. looked at mutations in *CFTR* in males with CBAVD presenting between 1992 and 2011 [14]. This study found that 75% of patients had at least one *CFTR* mutation and 53% had 2 mutations detected. Where only one mutation was found, the authors felt that a second mutation would be detectable if more extensive sequencing had been available [14]. Utilising the current techniques to scan the whole *CFTR* gene, it would be expected that only a small minority of mutations would be technically difficult to detect, for the reasons described above. As *CFTR* mutations in CBAVD follow the Mendelian pattern of autosomal recessive inheritance, if only one mutation is detected, it is accepted that there is a second mutation that has escaped detection [9]. This is an important concept when counselling patients and families and is discussed further later in the chapter.

Almost all the described CF mutations have been seen in CBAVD, being p.Phe508del, seen in 17% of reported series and the milder IVS8-5T allele seen in 25% [14]. In the series published by Phillipson et al., 24% of CF chromosomes carried the c.350G>A (previously known as R117H or Arg117His), similar to the 22% initially reported in CBAVD by Gervais [28, 29]. Compared with an incidence of 0.3% of the c.350G>A variant in classic CF, these data suggest that the c.350G>A allele is seen at an increased frequency in men with CBAVD.

IVS8-5T and the C.350G>A Alleles

Variations in the length of a polythymidine (polyT) tract within the splice acceptor site at the end of intron 8 of the *CFTR* gene lead to alternative splicing. This polyT tract in intron 8 is known as the invariant splice tract and documented as IVS8-(n)T

or simply (n)T, with (n) denoting the number of thymidines in the tract [19]. Alternative splicing results in 2 types of mRNA transcripts, one with and one without exon 9 of *CFTR* [19]. mRNA without exon 9 results in a *CFTR* protein that does not mature and cannot function as a chloride channel in the apical membrane epithelial cells [19]. The 3 common variants within the polyT tract contain 5, 7, or 9 thymidines and are known as the IVS8-5T, -7T, or -9T alleles, respectively [4]. The lower the number of thymidines in the polyT tract, the less efficient is the exon 9 splicing process [12]. The IVS8-5T allele can thus cause reduced levels of normal *CFTR* mRNA and has been implicated in the pathogenesis of CBAVD.

In CBAVD patients of European descent, the frequency of the IVS8-5T allele is four- to sixfold higher than in the general population [30, 31]. The same genotypes that include IVS-5T can be found in both CBAVD and non-CBAVD individuals, suggesting that the 5T allele has reduced penetrance [19]. The concept of the IVS8-5T allele being found in CBAVD in conjunction with a second *CFTR* mutation and potentially modifying the phenotype is supported by the work of Chillon et al. [30]. They studied 102 patients with CBAVD, none of whom had any additional clinical features of CF. They looked for known mutations and also analyzed non-coding DNA regions, looking at the polyT tract as described earlier. In the majority of their CBAVD patients, the presence of the IVS8-5T allele was strongly associated with the presence of a CF mutation on the other allele ($p = 0.0016$), suggesting that where an IVS8-5T allele is found, a careful search for a *CFTR* mutation on the other chromosome should be undertaken. Depending on the second mutation found the phenotype might vary from CBAVD to mild CF.

The incomplete penetrance of IVS8-5T is due at least in part to the number of nearby thymidine-guanine (TG) repeats that commonly number 11, 12, 13 or rarely, 15 TG repeats [4]. In contrast to the polyT tract, the higher the number of TG repeats, the lower the efficiency of splicing. Where the 5T allele is found in a patient with CBAVD, the adjacent TG repeat size should also be determined. Patients carrying 5T(TG)12 or 5T(TG)13 in cis (on the same allele), that is also in *trans* with a CF-causing mutation, may develop clinical features consistent with a mild form of CF and might therefore require further clinical evaluation and follow-up.

The specific mutation known as c.350G>A appears to be predominantly associated with CBAVD, as described earlier [24, 29, 30]. Phenotypic variability has been attributed to whether IVS8-T5 or -T7 is in *cis* with this mutation; i.e., c.350G>A (5T) being found in typical CF patients with pancreatic sufficiency and c.350G>A (7T) being associated with CBAVD [32].

A large study looked at 179 non-newborn French individuals (male and female) with c.350G>A in *trans* with a second *CFTR* mutation who were referred because of symptoms or family history [24]. Of the patients, 97% had intronic 7T in *cis* with c.350G>A, which is higher than that reported in studies of other populations [33]. Of the patients described, 89% were male (male: female 12.4:1) and 76% (136/179) had CBAVD [24]. Of 100 patients for whom clinical data were available, 66 had isolated CBAVD and 34/100 had CBAVD with additional symptoms—respiratory,

pancreatic, or nasopharyngeal. Of the CBAVD patients, 23% had respiratory features of moderate intensity, including asthma, isolated disseminated bronchiectasis, and recurrent bronchial infections. Two out of the 100 patients had pancreatic insufficiency. No significant differences were detected in clinical features or fertility depending on the class of the second mutation in *trans* with c.350G>A, with phenotypes ranging from isolated CBAVD to classical CF. Four healthy men in the study had c.350G>A in *trans* with a CF mutation and went on to spontaneously father children. This is further evidence that genotype–phenotype correlations are extremely difficult to make, and a wide range of CFTR-phenotypes can be found, even in patients with apparently similar genotypes.

Medical Management of Men with Biallelic *CFTR* Mutations

Guidelines suggest that where 2 classical *CFTR* mutations are found in a man with CBAVD, the diagnosis could be that of CF, rather than simply isolated CBAVD and that clinical evaluation in a CF center should occur [12]. Where one CF mutation is found to be in *trans* with IVS8-5T(TG)11, the diagnosis of *CFTR*-RD is confirmed and no further genetic investigations are required. If a *CFTR* classical mutation and the 5T allele are in *cis* with (TG)12 or (TG)13, clinical evaluation for features of CF is needed.

It is suggested that regular follow-up for CBAVD patients might be appropriate as 2 patients presented with pulmonary disease many years after their CBAVD diagnoses. Conversely, males with c.350G>A in *cis* with IVS8-5T and *trans* with a CF mutation, especially those with pancreatic or respiratory symptoms, should be offered fertility investigations.

Management of Men with no *CFTR* Mutations

If none or only one *CFTR* mutation or only the 5T allele is detected by a laboratory that is only offering targeted (level 1) screening, then more extensive testing (level 2 testing) should be requested. Best practice guidelines would suggest that clinical re-evaluation should occur at this point, including a sweat test and renal ultrasound [12].

Level 1 testing refers to a laboratory with the ability to screen for mutations occurring at a frequency of >1% in their local population. Level 2 refers to a laboratory that has the expertise to act as a consulting center for complex cases and the technical ability to detect all types of mutations using currently available techniques. Lewis-Jones et al. provide a list of mutations commonly screened for at both levels 1 and 2 [9].

In men with CAVD and negative *CFTR* mutation testing, complete renal tract imaging, often by renal and urinary tract ultrasound, is also recommended, due to data showing that approximately 20% of such males will have congenital urinary tract abnormalities or renal agenesis [15]. The CBAVD in this patient group is possibly caused by abnormal mesonephric duct development at an early embryonic stage [34]. Phillipson et al. found that 5 men with CBAVD in their study did not have any identifiable *CFTR* mutations and 2 of these 5 had unilateral renal agenesis [28]. There is a report by Meschede et al. of a patient with congenital unilateral absence of the vas deferens (CUAVD), renal tract anomaly, and a p.Phe508del mutation—in this case, the p.Phe508del is likely to be a co-incidental finding due to the high carrier frequency of this CFTR mutation in the general population [34, 35].

Genetic Counseling

If a diagnosis of *CFTR*-related infertility is confirmed in a man, the next step is to test his partner for a *CFTR* mutation. Genetic counseling prior to conception allows an estimation of the risk of a child having CF, based on both partners' mutation test results. Even if CF mutation testing in a female partner fails to reveal a mutation, there is still a chance that she could carry an unidentified mutation and the couple could go on to have a child with CF. Risk calculations using Bayesian theory are available to give an estimated residual carrier risk [9].

Couples at high risk of having a child with CF or CFTR-RD are those where the male partner has biallelic mutations and his partner carries a heterozygous *CFTR* mutation, or where he has had only one *CFTR* mutation identified and she carries a heterozygous *CFTR* mutation. When both partners in a couple carry at least one *CFTR* mutation, some couples may feel that an offspring risk of at least 25% is unacceptably high. They may therefore choose reproductive options such as sperm or egg donation, prenatal diagnosis (PND), preimplantation genetic diagnosis (PGD), or indeed adoption. If PGD is an option that a couple wishes to consider, a discussion will need to be had regarding the possibility of implanting embryos with a genotype that would predict a *CFTR*-RD phenotype. In some centers, couples may wish to eliminate the possibility of a phenotype of classical CF in a child but will be happy to *trans*fer embryos with, for example, c.350G>A and a classical CF mutation [24]. In addition, any member of a couple who is found to carry a *CFTR* mutation should be encouraged to inform their first-degree family members (siblings and parents). Under the English National Health Service (NHS) guidelines, at-risk family members can request genetic carrier testing via their general practitioner (GP), and if they are found to carry the familial mutation, their partner can also be tested. Those couples where both members are found to carry CFTR mutations can be referred to regional clinical genetics centers for genetic counseling, ideally prior to planning pregnancies.

In our center, increasing numbers of CF carrier couples are opting for referral for PGD, feeling that the 25% offspring risk in each pregnancy is unacceptably high. NHS funding is currently available for CF carrier couples who meet the personal medical eligibility criteria and who have no existing unaffected children. The ethics of testing an ostensibly healthy child of a carrier couple who has no clinical features but may have a genotype predisposing to mild or late-onset disease are complex, and some couples have self-funded cycles of PGD in this situation.

Fertility and Pregnancy Outcomes

Of men with CBAVD, 95–97% are infertile, manifesting as OA as described earlier [14]. Patrizio et al. showed a strong negative correlation between the severity of *CFTR* mutations and the success of IVF/ICSI using epididymal sperm taken from CBAVD patients [36]. The lowest IVF/ICSI success rate was seen in CBAVD males who were homozygous for the common p.Phe508del mutation associated with classical CF. Assisted reproductive technology (ART) was successfully performed in men with isolated CBAVD and *CFTR* mutations as reported by Thauvin-Robinet et al., but, although genotype-specific data were not provided, men with isolated CBAVD would not be expected to have a p.Phe508del homozygote genotype, so this ART success is perhaps not surprising [24].

The first pregnancy to a couple where the male partner had CBAVD was reported in 1987 [37]. Phillipson et al. looked at fertility and pregnancy outcomes in 27 azoospermic men, 25 with isolated CBAVD, and 2 with clinical CF [28]. CF mutation analysis in the 25 with CBAVD showed that 60% (15/25) had only one mutation identified, 20% were compound heterozygotes for known mutations, and 20% had no mutations identified. None were found to have the IVS-5T allele. All had satisfactory motile sperm recovered at surgical sperm aspiration, and 86% had supplementary sperm for cryopreservation, with 83% assessed as successfully thawed. Twenty-nine cycles with fresh sperm led to a fertilization rate of 76% of oocytes injected and 17% embryo implantation rate. Twenty-four cycles with cryopreserved sperm resulted in a fertilization rate of 69% and implantation of 20%, and 18 clinical pregnancies occurred with 14 live births. The authors concluded that *CFTR* mutations in a male partner do not compromise IVF treatment or opportunities for healthy birth.

Two pregnancies in this series were achieved after PGD: One couple was identified where both partners were p.Phe508del heterozygotes, i.e., a carrier couple with a 25% risk of a child with classical CF and one couple where the female partner was identified to be a compound heterozygote. This female compound heterozygote had a genotype of [c.1652G>A;IVS8-T7]/[c.350G>A;IVS8-T7], the c.1652G>A variant conferring a risk of classical CF in her offspring, although she was asymptomatic as would be expected with this genotype. Her partner had 1 p.Phe508del mutation identified, and therefore, the couple had a risk of at least

25% of their embryos being c.1652G>A/p.Phe508del and having classical CF. They therefore opted for PGD by blastomere biopsy with PCR amplification.

Two to three percent of males with CF and *CFTR*-RD are fertile [38]. The patient reported by Dreyfuss et al. was homozygous for the 3849+10kbC>T mutation and had extensive bronchiectasis but normal sweat electrolytes and was pancreatic sufficient. Sperm biopsy was consistent with fertility, and indeed, his children were confirmed to be heterozygote carriers for the mutation. Passero et al. reported males who were compound heterozygous for 3849+10kbC>T/3659delC mutations; they had respiratory features consistent with CF but successfully and spontaneously fathered children [39].

Screening of Gamete Donors

All men in the UK are screened for *CFTR* mutations prior to sperm donation, given the high carrier risk in Caucasian populations [9]. When both sperm donors and recipients are screened for mutations in *CFTR*, the incidence of babies born with CF is considerably reduced, to approximately one in 230,000. When a severely oligospermic or azoospermic male with CBAVD is found to have negative routine *CFTR* screening, there is a tendency not to proceed with screening the female partner, but Lewis-Jones et al. suggest this is fundamentally incorrect and leaves the couple with a potential residual risk of having a child with CF [9].

In actuality, where a CBAVD male has tested negative on routine level 1 screening and his female partner is a carrier of a *CFTR* mutation, the male needs testing for rare (level 2) *CFTR* mutations. Pragmatically, the safest approach is that both partners should have stepwise testing of *CFTR* before ART is undertaken, similar to the approach described earlier for sperm donors and recipients. In resource-limited settings, the female partner only could be screened first. Then only if the female is found to be a carrier does the male partner need screening, and if both are negative, the maximum residual risk for a child with CF or CBAVD is one in 960 [9]. It must be remembered that this approach would fail to detect mutations in certain populations such as Southern Europe and more extensive testing may be required. Families with a previous child with CF are increasingly asking for referrals to discuss PGD, so definitive risk assessments and molecular characterization should occur for all families at risk of a child with a CFTR-related phenotype.

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Chapter 11
Sperm Retrieval Techniques

Chak-Lam Cho and Ashok Agarwal

Introduction

The collection of sperm from the male genital tract was first described in 1985 [1]. But the procedures of sperm retrieval become an integral part of the management of azoospermia only after the report of a successful pregnancy by using testicular sperm extraction followed by intracytoplasmic sperm injection (ICSI) in 1993 [2]. While testicular sperm are retrieved from men with nonobstructive azoospermia (NOA), sperm may be retrieved from either the epididymis or testis in men with obstructive azoospermia (OA).

Cochrane meta-analysis has determined that there is insufficient data from trials to recommend any particular surgical sperm retrieval technique for either OA or NOA [3]. The complex interplay between male and female factors, and sperm retrieval and artificial reproductive technology (ART) means the management of infertile couples should be individualized.

In this chapter, we describe the preoperative preparation and postoperative care for patients undergoing sperm retrieval procedures. The principles of selection among different sperm retrieval techniques for patients with OA and NOA are illustrated by 2 clinical scenarios.

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Preoperative Preparation

Patients are instructed to withhold anticoagulants and antiplatelet medications for a week before the procedure. Abstinence from ejaculation for 2–3 days prior to the procedure is advised. Blood tests including screening of infectious diseases are performed. Laboratory tests for infectious disease status may consist of hepatitis, syphilis, and human immunodeficiency virus (HIV) and may vary between centers. A quarantine cryopreservation tank may be required in case of positive test results.

The vast majority of sperm retrieval procedures are performed on an outpatient basis. Shaving or clipping of the surgical site can be performed 1 day before or on the day of surgery [4]. Intravenous antibiotic with coverage of Gram-positive organisms is administered at least 30 min before skin incision except for percutaneous procedures. A bench microscope with appropriate containers and transport media should be available in the operating room for intraoperative examination of specimens. An experienced embryologist should be present, if necessary.

General anesthesia is generally preferred if the procedure involves the use of an operative microscope. Local anesthesia may be employed for percutaneous procedures, particularly in the outpatient setting. However, many patients reported significant discomfort and anxiety during percutaneous sperm retrieval procedures with spermatic cord block alone [5]. The co-administration of intravenous sedation would offer patients the additional benefits of an anxiolytic and possibly amnesia. Patients have reported greater satisfaction with the addition of intravenous sedation especially for bilateral or longer procedures [6].

Case Scenarios

Case 1

A 50-year-old gentleman, who had a vasectomy 15 years ago, wishes to have another child. His wife is 38 years old with a normal evaluation. They have 1 child together but also a history of 3 miscarriages. Testes measure 20 cc in volume bilaterally. Both epididymides are mildly prominent. The couple decides for sperm retrieval and ART instead of reconstructive surgery.

Diagnostic testing for post-vasectomy patients before sperm retrieval is generally not necessary. Hormonal evaluation with serum follicle-stimulating hormone (FSH) and testosterone is performed if there is clinical suspicion of impaired spermatogenesis.

Options of Sperm Retrieval Procedures

Sperm may be retrieved from epididymis or testis in azoospermic men after vasectomy. Epididymal sperm retrieval is the most commonly performed procedure in this situation. The procedure can be performed by percutaneous or open approaches. Percutaneous epididymal sperm aspiration (PESA) can be performed under local anesthesia, and an operative microscope is not required. It may be performed in an office setting. Patients recover quickly after the procedure with low complication rates. A needle is advanced percutaneously into the epididymal tubules. The needle is advanced in and out gently with negative suction force applied via a 30 cc or 60 cc syringe. Epididymal fluid is aspirated. Around 0.1 mL of fluid is usually obtained per aspirate [7]. The procedure is repeated at different sites from cauda to caput epididymis until an adequate number of motile sperm are retrieved. Since PESA is a blind procedure, multiple attempts may be required to obtain good quality sperm. Aspirates from cauda are often rich in senescent spermatozoa, debris, and macrophages. The phenomenon of better quality sperm at the proximal reproductive tract in men with chronic obstruction has been termed "inverted motility" [8]. It may be rational to start the procedure from corpus epididymis toward caput since vasectomy was performed 15 years ago. The concern about the detrimental effect of PESA on subsequent reconstructive microsurgery is not valid [9].

Epididymal sperm can also be acquired by an open approach with microscopic epididymal sperm aspiration (MESA). MESA involves incision of the epididymal tunica and inspection of the epididymal tubules. Dilated epididymal tubules with clear contents are punctured or incised, and the fluid is collected. A single MESA procedure usually enables retrieval of a great number of motile sperm [10], which are usually sufficient for cryopreservation for multiple subsequent in vitro fertilization (IVF) cycles [11]. The open procedure allows better hemostasis and decreased risk of hematoma formation compared to PESA [12]. Also, the contamination of the sample by red blood cells is minimized. The risk of scarring and epididymal obstruction after MESA is likely lower compared to PESA due to targeted aspiration of individual tubules under direct microscopic vision, and incised tubules can be repaired. MESA has been modified to combine advantages of percutaneous technique with precision of microsurgical procedure. The epididymis is brought anteriorly and examined via a 1–2-cm scrotal incision during the procedure of "mini-MESA" [13].

Testicular sperm can be retrieved by testicular sperm aspiration (TESA) percutaneously from men with OA. The testicular parenchyma is aspirated by fine needle, large-diameter needles, or tissue-cutting biopsy needles. Location of sperm aspiration matters little in terms of successful sperm retrieval. Sperm in obstructed testes is found throughout the parenchyma [14]. An entry point starting at the superior testicular pole and passing inferiorly and obliquely may carry less risk of vascular injury. Conversely, testicular sperm extraction (TESE) is rarely employed in men with OA. The pros and cons of various sperm harvesting techniques for OA is summarized in Table 11.1.

Table 11.1 Advantages and disadvantages of sperm retrieval techniques for obstructive azoospermia

	Advantages	Disadvantages
PESA	• Fast • Low cost • Possibly office/outpatient procedure • Minimal recovery and morbidity • Repeatable • No microsurgical skill and instruments required	• Few sperm retrieved • May not retrieve adequate sperm for cryopreservation • May cause epididymal obstruction at puncture sites • Risk of hematoma formation
MESA	• Ample sperm retrieved • Excellent chance of sperm cryopreservation • Decreased risk of hematoma	• Increased cost and anesthetic/operating time • Microsurgical skill and instruments required • Surgical exploration required with longer postoperative recovery
TESA	• Fast • Low cost • Possibly office/outpatient procedure • Minimal recovery and morbidity • Repeatable • No microsurgical skill and instruments required	• May not retrieve adequate sperm for cryopreservation • Risk of hematoma formation • Risk of testicular atrophy
TESE	• Fast • Repeatable • No microsurgical skill and instruments required	• Increased cost and operating time • Surgical exploration required with longer postoperative recovery • Risk of testicular atrophy

PESA percutaneous epididymal sperm aspiration; *MESA* microsurgical epididymal sperm aspiration; *TESA* percutaneous testicular sperm aspiration; *TESE* conventional testicular sperm extraction

Selection and Results of Sperm Retrieval Technique in Post-vasectomy Patients

The sperm retrieval rate (SRR) in patients with OA is high and ranges from 90 to 100% [15]. Successful PESA has been reported in 78.0% of the cases, and subsequent percutaneous testicular retrievals are successful in the vast majority of failed epididymal sperm retrievals. The cumulative success rate of percutaneous approaches in OA patients reaches 97.3% irrespective of the cause of obstruction [16]. A low SRR of around 20% has been reported when an epididymal cyst is present, which is a common finding after vasectomy [17, 18]. In this patient population, however, subsequent sperm retrieval by TESA or TESE still carries a high success rate.

The history of multiple miscarriages without an identifiable female factor in our clinical scenario also needs to be investigated and may have implication on the choice of sperm retrieval technique. The impact of paternal factors on reproductive

outcomes is increasingly being recognized. Sperm deoxyribonucleic acid (DNA) fragmentation has been widely studied in recent years and has been increasingly associated with recurrent pregnancy loss particularly in the setting of ART [19]. The association between aging and loss of DNA integrity [20] is particularly worrisome in our patient. Sperm DNA fragmentation (SDF) testing may be diagnostic in identifying the etiology of recurrent miscarriage, especially after ART failure. Testing may also provide prognostic information on ART outcomes for the couple. There is evidence to show that high SDF is associated with increased risk of pregnancy loss and decreased live birth [21]. Treatment strategies, including oral antioxidants and sperm selection, can be considered in case of elevated SDF levels. Testicular sperm retrieval with TESA or TESE may be preferable in patients with high SDF since the incidence of DNA fragmentation is markedly lower in testicular sperm [22, 23].

It is rational to start retrieval of epididymal and/or testicular sperm percutaneously (i.e., PESA ± TESA) in our patient with expected high SRR approaching 90–100% [24]. Percutaneous sperm retrieval provides the advantages of minimal invasiveness with low complication rate. The procedure can be performed in the office setting under local anesthesia without the use of operative microscope and microsurgical technique. PESA should start from corpus epididymis toward caput in view of the phenomenon of inverted motility. MESA should be considered if our patient desires a single sperm retrieval procedure and cryopreservation for multiple subsequent ART cycles [11, 25]. Retrieval of testicular sperm should be considered in the presence of epididymal cyst or high SDF.

Artificial Reproductive Technology Outcomes in Men with Obstructive Azoospermia

Pregnancy success rates utilizing epididymal sperm from patients with OA in intrauterine insemination (IUI) [26] and in vitro fertilization [1] have been reported. Good oocyte fertilization and pregnancy rates in ICSI have been achieved with epididymal sperm. Fertilization, clinical pregnancy, and live birth rates of 60, 50, and 35%, respectively, can be achieved [27]. The use of testicular sperm from men with OA in ICSI is also associated with similar high pregnancy rates [28]. The source of sperm and retrieval modality does not affect outcome of ART in our patient [29, 30]. ICSI outcomes using fresh or frozen-thawed sperm retrieved from men with OA are also comparable [20, 31].

Sperm quality in OA patients is generally high. If sperm quality or quantity from the epididymis is poor, consideration should be given to TESE. Cryopreservation and use of frozen-thawed sperm in ART will not compromise the reproductive outcomes in our patient.

Case 2

A 30-year-old gentleman has infertility and azoospermia on semen analysis. His 28-year-old wife has a normal evaluation. Testicular volume is 8 cc bilaterally with palpable vasa deferentia on physical examination of the patient. A grade 3 left varicocele was revealed on physical examination. Serum FSH and testosterone levels are 30 IU/L and 200 nmol/L, respectively. Testicular sperm retrieval has been attempted previously with no sperm retrieved, and there is no further detail available.

While retrieval of good quality sperm from men with OA is very likely, sperm retrieval success rates in men with testicular failure and NOA is much lower. Donor sperm insemination and child adoption were the options left to men with NOA a few decades ago. The finding of heterogeneous "patchy" spermatogenesis within the testes of approximately one-third of men with NOA on a single diagnostic biopsy provides the rationale in the management of NOA by sperm retrieval [32]. Despite the severely impaired spermatogenesis with inadequate sperm production to reach the ejaculate, sperm can be demonstrated within the testes in at least 60% of men with NOA in a more recent study [33]. Testicular sperm retrieval combined with ICSI in our patient offers the chance for the patient to father his own biologic children.

Preoperative Investigations and Optimization

Meticulous microscopic examination of the pellet is necessary to determine whether a semen sample is truly azoospermic. It is shown that sperm are identified in up to 35% of men who are thought to have NOA during an extended examination of a centrifuged specimen [34]. The definitive diagnosis of NOA relies on histological confirmation. However, a clinical diagnosis based on history, small testicular volume, and flat epididymides on physical examination, elevated serum FSH levels, and azoospermia on semen analysis can be made in many cases.

Diagnostic testicular biopsy remains the gold standard in differentiation between OA and NOA. However, the small samples obtained from diagnostic biopsy are unlikely to be representative since both testicular histology and sperm production are heterogeneous within the seminiferous tubules. Currently, many centers perform testicular biopsy for histology at the time of sperm acquisition. A separate procedure of testicular biopsy is not regarded as mandatory before sperm retrieval procedures by many male fertility specialists.

Karyotyping and Y-chromosome microdeletion (YCMD) testing typically identify the etiology of impaired spermatogenesis in 15–20% of NOA patients, and up to 17% of TESE candidates are found to have abnormal genetic evaluation [35]. YCMDs are more commonly detected in patients with lower sperm production. Ten percent of azoospermic men are noted to have YCMD, while no microdeletion is

detected in men with sperm counts more than 5×10^6 [36]. It is now possible for the transmission of defective genetic material to offspring with advent of ICSI and sperm retrieval. It is therefore advisable to have genetic evaluation before sperm retrieval. Results of genetic tests have been shown to alter the choice of treatment in 21% of infertile couples [35]. Donor sperm, adoption, and embryo biopsy are some options elected by patients after genetic counseling. Apart from genetic evaluation, elevated serum FSH level is one of the clinical features of men with NOA. The prognostic value of hormonal and genetic evaluation on sperm retrieval will be discussed later in this chapter. Imaging modalities are generally not indicated for the management of NOA patients unless there are abnormalities on physical examination.

Spermatogenesis should be optimized for at least 3 months prior to sperm retrieval. Any reversible causes should be corrected including avoidance to gonadal toxins. The role of varicocelectomy in our patient with NOA is not well defined. Most patients have no return of sperm to the ejaculate and require sperm retrieval despite repair of varicocele [37]. Varicocelectomy does not influence subsequent SRR in men with NOA and clinical varicoceles. The beneficial effect of varicocelectomy may take 6 months or longer to appear and, therefore, may not be a sensible choice for our patient. Hormonal disturbances, including compromised serum testosterone and increased estradiol levels, are common among men with NOA [38]. Testosterone-to-estradiol ratio (TE ratio) is commonly used clinically as an expression of the overall androgen and estrogen balance. The mean TE ratio in fertile controls is significantly higher compared to men with severe infertility [38]. Increased aromatase activity of the testes may contribute to the phenomenon [39]. By directly limiting estrogen feedback to the pituitary gland, aromatase inhibitors increase production of FSH and luteinizing hormone (LH). The correction of the endocrinopathy may enhance endogenous intratesticular testosterone levels and thus spermatogenesis. Both steroidal (testolactone) and nonsteroidal (anastrozole) aromatase inhibitors raise serum testosterone levels and correct TE ratios effectively [40]. A TE ratio of less than 10 is proposed as the cutoff to initiate treatment. Although significant improvements in the hormonal profile and semen parameters have been demonstrated in oligozoospermic men treated with testolactone, there are no studies that have demonstrated a return of sperm to the ejaculate in azoospermic men with treatment [38]. The use of aromatase inhibitors in men with NOA remains off-label. The correlation between hormone manipulation and fertility benefits remains to be defined by randomized controlled studies.

In summary, karyotyping and YCMD testing should be performed in our patient before sperm retrieval procedures. The test results carry important prognostic value. Varicocelectomy as an adjunct before sperm extraction has no evidence to improve SRR. Testing of estradiol level may be considered, and treatment initiated with TE ratio less than 10. However, the current evidence of correction of endocrinopathy with aromatase inhibitors in our patient with NOA is weak.

Procedures in Men with Previous Failed Sperm Retrieval

Failure of previous sperm retrieval does not deter further attempts in our patient. The characteristic of patchy foci of sperm production in men with NOA renders a single biopsy inadequate for identification of sperm most of the time. Multiple biopsies are essential for the successful sperm retrieval in men with NOA. Only 23% of men have sperm identified on the first biopsy, and up to 14 biopsies may be required to locate sperm in a single procedure of open testicular biopsy from 1 or both testicles [41]. Repeating testicular biopsy or testicular sperm aspiration (TESA) may be a less favorable option in view of the low SRR. Microdissection testicular sperm extraction (mTESE) after previous failed TESA or TESE procedures is more commonly practiced and studied. Patients who had 1 or 2 prior biopsies per testis have an SRR of 50% by mTESE. SRR decreases to 22% if 3 or more previous biopsies were performed per testis. This is compared to 52% of SRR with mTESE in patients who have no prior testicular surgery [42]. The minimal impact on subsequent SRR by 1 or 2 prior testicular biopsies strongly suggests that random testicular biopsies commonly miss areas of sperm production. The chance of sperm identification on repeated mTESE is 33% even when no sperm is found on the first mTESE [43]. Data show that mTESE achieves reasonable SRR after failed testicular biopsy, TESA, TESE, and mTESE in the hands of an experienced infertility surgeon. Repeated mTESE appears a viable option for our patient.

A 6-month interval between sperm retrieval procedures is recommended to our patient. This recommendation is based on the concept that spermatogenesis can be adversely affected by postoperative changes and sperm production may take 3 months to be fully restored. Although clinical data on the effect of the time interval between sperm retrieval procedures and SRR are lacking, the suggestion of a 6-month interval is supported by circumstantial evidence. It is found that 82% of abnormal sonographic findings of the testes at 3 months after TESE procedures resolve by 6 months [44]. The incidence of ultrasound findings suggestive of hematoma decreases from 5 to 7.5% and 12 to 2.5% at 1 and 6 months after conventional TESE and mTESE, respectively, suggesting that at least 6 months is needed for most of the testes to fully recover after sperm retrieval procedures [45]. However, the varying degree of testicular damage caused by different sperm retrieval procedures indicates that the optimal timing to repeat sperm retrieval procedures should be individualized. Serial ultrasound imaging of the testes may be helpful in defining the optimal time interval. While the majority of ultrasound abnormalities resolve by 6 months, endocrine function and serum testosterone level may take up to 18 months to recover [46]. The question remains unanswered, and the optimal time interval between sperm retrieval procedures is yet to be defined by further research.

Sperm Retrieval Procedures for Men with Nonobstructive Azoospermia

The testis is the only sperm source for our patient. There are several options available for testicular sperm retrieval: (1) TESA, (2) conventional TESE, (3) mTESE, and (4) testicular mapping. TESA attempts to retrieve sperm by percutaneous technique. The procedure can be performed under cord block and local anesthesia when a fine needle is used. The low SRR renders percutaneous procedures uncommon [47, 48]. TESA is not recommended as the primary procedure of sperm retrieval for men with NOA except when used in conjunction with testicular mapping. It has been shown that percutaneous procedures are less effective than open testicular biopsy in obtaining sperm [33, 49].

TESE and mTESE are open testicular biopsy techniques and are more commonly performed in men with NOA. Multiple biopsies are usually employed to locate sperm during conventional TESE [41]. Conventional multiple biopsy TESE achieves up to 50% SRR [50]. However, it carries the risk of damage to the testicular blood supply. Complete testicular devascularization has been reported after multiple biopsies.

Since the introduction of mTESE in 1999 [33], the procedure has gained popularity due to several advantages over conventional TESE. The use of a microscope allows identification of subtunical blood vessels and decreases the risk of damage to the testicular blood supply [51]. A higher SRR of 45–65% is associated with mTESE compared to 30–45% with conventional TESE [33, 47, 51]. Moreover, mTESE is more effective in recovering sperm from men with testicular volume of less than 10 mL [52]. Larger quantity of sperm is obtained during mTESE with less testicular tissue removed. An average of 160,000 spermatozoa are obtained in samples that weigh 9.4 mg during mTESE, compared to 64,000 spermatozoa yielded by 720 mg of testicular tissue from conventional TESE [33]. However, it was concluded in a systematic review that mTESE performs better than conventional TESE only in cases showing Sertoli-cell-only pattern on histology where tubules containing foci of active spermatogenesis can be identified by the microscopic appearance of larger and more opaque tubules [53].

mTESE also has the lowest complication rates compared to other sperm retrieval techniques [53]. mTESE results in less intratesticular reaction than conventional TESE despite the wide equatorial incision along the tunica albuginea and extensive dissection. The achievement of complete hemostasis during mTESE results in less acute and chronic sonographic changes on scrotal ultrasound. Less postoperative pain after mTESE has been reported due to less retraction of tunica albuginea and compression of testicular parenchyma [54].

Despite the advancement in sperm retrieval techniques, lasting effects on testicular function after testicular sperm extraction should not be overlooked. Serum testosterone levels drop by 20% of preoperative levels at 3–6 months after sperm retrieval procedures and are not completely recovered at 18 months postoperatively [46]. It also has been reported that mTESE leads to reduction in serum testosterone

levels and increase in FSH and LH levels [55]. Histologic studies of the testes after sperm extraction procedures reveal a 7 and 5% decrease in seminiferous tubule volume and germ cell density, respectively [56].

Another option for obtaining sperm from our patient with NOA is testicular mapping. It consists of systematic fine needle aspiration (FNA) following a 22-site template of bilateral testes. Further management is stratified by the test results. Patients who have no sperm identified are offered the options of adoption and donor insemination, and attempt to use a sperm retrieval procedure is generally not recommended in expert centers. On the other hand, a directed sperm retrieval procedure will be offered in the presence of sperm. The location and quantity of sperm identified on mapping guide the subsequent sperm retrieval procedures. Testicular mapping is an outpatient procedure performed under local anesthesia. The procedure is well tolerated, and patients usually resume normal activity within a day [57]. An early study has demonstrated the potential use of FNA to identify sperm in men with NOA with 2–3 samples from each testis [58]. The role of FNA is further supported by a report of 60% SRR in men with NOA with up to 15 samples from each testes, but the quantity is insufficient to inject all a partner's ova in most cases [59]. Therefore, testicular mapping/FNA as the sole sperm retrieval procedure is not recommended. The optimal number of sites of diagnostic aspiration remains unclear. Despite the advantage in avoiding or minimizing the invasiveness of sperm retrieval procedures, the wide application of testicular mapping is hindered by the significant cytologic experience required in identifying sperm in a smear of aspirated seminiferous tubules.

Subsequent sperm retrieval is executed from the least to most technically demanding procedures in the sequence of TESA, conventional TESE, and mTESE based on the map. It has been demonstrated that sufficient sperm for injection of all available oocytes can be retrieved in 95% of cases [60]. Bilateral procedure was only required in 22% of patients. Complex sperm retrieval with mTESE was performed in 23% of men, while the majority had sperm acquired by TESA and TESE [60]. It is of note that the high SRR was reported from patients with positive FNA results to begin with. Currently, there is no head-to-head studies comparing the different strategies of mTESE and testicular mapping ± sperm retrieval. The advantages and disadvantages of various sperm retrieval techniques in men with NOA are presented in Table 11.2.

The importance of intraoperative specimen handling in increasing sperm yield has been addressed. The mechanical disruption of individual tubules by aggressive mincing in the medium and repeated passage of testicular suspension via angiocatheter increases sperm yield by up to 300-fold [61]. The procedure of sperm retrieval can be terminated once sufficient sperm are identified in the operating theater by surgeon or embryologist under microscope. The increased efficacy in sperm identification prevents unnecessary damage to the already compromised testis of our patient.

In summary, repeating sperm retrieval at least 6 months after the previous attempt is a rational approach for our patient. mTESE seems the preferred technique

Table 11.2 Advantages and disadvantages of sperm retrieval techniques for nonobstructive azoospermia

	Advantages	Disadvantages	Sperm retrieval rates (%)
TESA	• Fast • Low cost • Possibly office/outpatient procedure • Minimal recovery and morbidity • No microsurgical skill and instruments required	• May not retrieve adequate sperm for injection of all retrieved oocytes • Risk of hematoma formation • Risk of testicular atrophy	5–10
TESE	• No microsurgical skill and instruments required	• Surgical exploration required with longer postoperative recovery • Risk of testicular atrophy	30–45
mTESE	• Thorough examination of testicular parenchyma • Reduced risk of damage to testicular blood supply • Less testicular tissue removed • Less adverse effect on testicular function	• Increased cost and operating time • Surgical exploration required with longer postoperative recovery • Microsurgical skill and instruments required	45–65
Testicular mapping (± sperm retrieval)	• Possibly office/outpatient procedure • Minimal recovery and morbidity • No microsurgical skill and instruments required • Avoid morbidities associated with sperm retrieval procedures for patients with no sperm identified on testicular mapping • Potentially reduce the invasiveness of the subsequent sperm retrieval procedure	• Significant cytologic experience required • Some patients are subjected to 2 procedures • Possible false negative despite extensive systematic fine needle aspirations	95[a]

TESA percutaneous testicular sperm aspiration; *TESE* conventional testicular sperm extraction; *mTESE* microdissection testicular sperm extraction
[a]Patients with sperm identified on testicular mapping

and has been more widely studied as the procedure after failed TESA/conventional TESE/mTESE attempts and showed promising results. The technique has also been suggested to be particularly useful for our patient with small testicular volume. mTESE may have less of a detrimental impact on testicular function. There is more rapid recovery of hormonal profile and resolution of sonographic abnormalities after mTESE. Meticulous specimen handling intraoperatively is of paramount importance in maximizing the sperm yield. The alternative of testicular mapping ± sperm retrieval can be considered if significant cytologic expertise is available.

Prognostic Factors

The success of surgical sperm retrieval in men with NOA is variable. Early studies in identifying prognostic factors for successful sperm retrieval have been disappointing. Clinical features, including testicular volume, history of ejaculated sperm, serum FSH or inhibin levels, etiology of NOA, and biopsy histology, do not predict success of sperm retrieval procedures [62, 63]. More recent data suggest that YCMD and histopathologic diagnosis are the most promising predictive factors. The presence of AZFc in azoospermic men is considered a favorable factor associated with a SRR of 71.4% compared to 48.8% retrieval rate in patients with idiopathic azoospermia [64]. The clinical pregnancy rates per IVF cycle involving sperm retrieval from men with AZFc microdeletion are comparable to that of unaffected individuals [65]. On the other hand, sperm retrieval is universally unsuccessful in all patients with complete AZFa or AZFb deletions [66].

Histopathologic diagnosis may be helpful in predicting treatment success in case prior diagnostic biopsy has been performed. The most advanced stage on biopsy, but not the predominant stage, is considered as the predictive factor [63]. It has been shown that sperm are identified in 81, 44, and 41% of patients with hypospermatogenesis, maturation arrest, and Sertoli-cell-only, respectively, by using mTESE [46]. Correlations between SRR and histopathologic diagnosis are also demonstrated with standard open testicular biopsy [67] and testicular FNA techniques [59]. A study suggested that the presence of Sertoli-cell-only on biopsy as the most advanced pattern in men with at least 1 prior failed sperm retrieval is associated with lower SRR [42]. Other factors have been suggested to have prognostic value on SRR as well. The role of serum FSH level as a predictive factor of successful sperm retrieval is less well defined. One study has demonstrated that a cutoff level of serum FSH > 20 IU/L predicts successful sperm retrieval with open biopsy methods [68]. Conversely, other studies have demonstrated that serum FSH levels are less relevant for predicting success of mTESE. Patients who have serum FSH of 15–30 IU/L, 30–45 IU/L, or greater than 45 IU/L all have similar SRRs [69].

The negative effect of prior biopsies on conventional TESE is suggested by 56% SRR in men who underwent no prior biopsy compared to 23% SRR for those who had 3–4 biopsies per testis [41]. The phenomenon may be explained by scarring and parenchymal fibrosis as a result of devascularization by multiple biopsies. Prior success in sperm retrieval predicts good SRR on repeat procedures. The SRR reaches 96% on repeated mTESE following prior successful retrieval. On the other hand, the SRR drops to 33% if sperm is not found on previous mTESE [43].

It also has been proposed that the response to aromatase inhibitor in men with Klinefelter syndrome predicts the results of sperm retrieval [70]. Whether the result can be extrapolated to other non-Klinefelter syndrome men with NOA and low TE ratio is unknown. Table 11.3 is a summary list of the possible prognostic factors for sperm retrieval in men with NOA.

Therefore, obtaining the details of the previous sperm retrieval procedure is of paramount importance in addition to YCMD test results in our patient. Although a

Table 11.3 Prognostic factors for successful sperm retrieval in men with nonobstructive azoospermia

Y-chromosome microdeletion	Complete deletion of AZFa or AZFb are extremely poor prognostic factors Presence of AZFc is associated with sperm retrieval rate of approximately 70%
Histopathologic diagnosis	Sertoli-cell-only pattern is generally associated with lower sperm retrieval rate compared to hypospermatogenesis and maturation arrest
Serum follicular-stimulating hormone (FSH)	FSH > 20 IU/L signifies remote chance of sperm retrieval with conventional multifocal testicular sperm extraction (TESE) Serum FSH does not predict sperm retrieval rate during microdissection testicular sperm extraction (mTESE)
Previous testicular biopsies	More than 2 prior biopsies per testis is associated with reduced chance of sperm retrieval
Results of prior sperm retrieval	Prior success in mTESE is associated with 96% sperm retrieval rate in repeated procedure Repeating mTESE in patients with previous failed attempt is associated with 33% sperm retrieval rate
Response to aromatase inhibitors	The role of aromatase inhibitor in non-Klinefelter syndrome men is unclear

prior failure of sperm retrieval predicts lower success on subsequent procedures, other information such as the surgical technique of previous attempts and histopathologic diagnosis also carries prognostic value.

Fresh Versus Cryopreserved Retrieved Testicular Sperm

There has been considerable debate between using fresh versus frozen testicular sperm for ART in men with NOA. A meta-analysis concludes that fertilization rates, clinical pregnancy rates, and ongoing clinical pregnancy rates do not differ between groups using fresh or cryopreserved testicular sperm from men with NOA [15]. Some authors also suggest cryopreservation of retrieved testicular sperm followed by ICSI later in order to avoid unnecessary ovarian stimulation of the female partner [71]. But there is a concern of using cryopreserved testicular sperm for ICSI based on the finding that only 33% of testicular samples from men with NOA show documentable viability after freeze-thaw [72]. Currently, many fertility specialists prefer fresh to freeze-thawed testicular sperm. Coordinated IVF cycles and sperm extraction procedures are required in order to use fresh testicular sperm. Fresh testicular sperm has a high viability rate approaching 90% despite its low motility. Injection of nonmotile fresh testicular sperm during ICSI yields a high fertilization rate [73]. It is now recognized that the motility of retrieved testicular sperm remains stable or increases with incubation in vitro for 24–48 h [74]. This has simplified the timing of procedures on infertile couples, and testicular sperm can be retrieved 1–2 days before ICSI.

Postoperative Care

Postoperative care differs between centers and surgeons. Ice packs may be applied intermittently to the scrotum for 24–48 h. Patients are strongly advised to wear briefs or a scrotal supporter until edema and pain subside. Scrotal swelling, wound ecchymosis, and discomfort usually subside in approximately 7 days. Normal daily activities can be resumed on the next day after percutaneous sperm retrieval and 3 days after open procedures. Men can begin showers after 24 h. Strenuous exercise should be avoided for 7–10 days. No sexual activity is recommended for 3–7 days. Antibiotic after the procedure is not necessary and not routinely prescribed [75], but some surgeons may prefer empirical oral antibiotic for 3–5 days. Pain medication is used as needed. Common prescription including narcotics or nonsteroidal anti-inflammatory medications is usually adequate for pain control.

Conclusion

The acquisition of satisfactory surgical sperm retrieval technique is essential for all male fertility specialists. Currently, there is insufficient evidence from randomized trials to recommend any particular procedure for both obstructive and nonobstructive azoospermia. A variety of procurement procedures are available, and the choice of techniques varies among centers. The formulation of a protocol for sperm retrieval at a particular center largely depends on the expertise and equipment available. The collaboration and discussion among male fertility specialists, ART specialists, and embryologists is essential. Choosing the right surgical approach can only be made with a thorough understanding of the pros and cons of each sperm extraction technique.

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Chapter 12
Varicocele: Surgical Intervention Versus Assisted Conception

Nicholas N. Tadros and Edmund Sabanegh Jr.

Introduction

Clinical Vignette

A 34-year-old man presents to your clinic for infertility. He has a 4-year-old daughter and has been trying to have a second child with his wife for the last 18 months. He brings with him a semen analysis that shows oligoasthenoteratospermia (OAT). He states that he has noticed a small bulge in his left scrotum for the last few years that does not cause discomfort. His examination is significant for a left grade 2 varicocele, and an ultrasound of his scrotum reveals a left varicocele that enlarges with Valsalva. He is wondering whether there is anything that can be done to help him and his wife conceive. They would like to avoid in vitro fertilization if possible.

Male factor infertility is implicated in 50% of couples being evaluated for infertility. Varicoceles, defined as abnormally dilated scrotal veins, are thought to be the most common attributable cause of infertility in the male [1]. Their pathophysiology is still poorly understood, despite the first varicocele being diagnosed in the first-century CE by Celsus [2]. Most studies are retrospective or case controlled, and there are few well-done randomized studies [3]. With the advances in assisted reproductive techniques (ARTs), some physicians question the need to treat

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varicoceles at all. Though the debate continues, many studies have shown that treatment of varicoceles in well-selected patients can indeed improve a man's fertility and chance of pregnancy. We present a review of the evidence for the role and treatment of varicoceles in the infertile male and its effects on the need for assisted reproductive techniques.

Incidence

In the largest population study on varicoceles to date, the World Health Organization (WHO) found varicoceles in 11.7% of the men with normal semen parameters [4], but up to 42% of men with primary infertility [5] and up to 81% of men [6] with secondary infertility (i.e., men who have previously fathered a child but are now unable to do so). Interestingly, in a small study of men with varicoceles, 57% of their first-degree relatives (particularly brothers) had palpable varicoceles themselves [7].

Pathophysiology

Anatomy

While the semen abnormalities caused by varicoceles are likely multifactorial, the development of the varicocele itself is assumed to be more straightforward and is caused by structural abnormalities of the vascular supply of the testis. The arterial supply to the testis is derived from 3 main sources: the testicular artery, which supplies about two-thirds of the blood flow, and the vasal and cremasteric arteries, which supply the rest [8]. This arterial anatomy is important when surgical treatment of a varicocele is planned and will be discussed in further detail later in this chapter.

The venous drainage of the testis is more variable but classically starts in the scrotum as the pampiniform plexus and eventually drains into the testicular (internal spermatic) vein. This vein then drains into the renal vein on the left, or directly into the inferior vena cava (IVC) on the right. The pampiniform plexus is also drained via the vasal vein, which drains into the vesicular veins and the cremasteric veins, which eventually drain into the inferior epigastric vein.

It is theorized that varicoceles result when there is too much back pressure in this drainage system. The left testicular vein is 8–10 cm longer than the right and inserts into the left renal vein at approximately a 90° angle, whereas the right testicular vein inserts more obliquely into the vena cava. These factors are believed to provide the right testicular vein with less turbulent flow and back pressure, which results in a substantially lower incidence of right-sided varicoceles (1–2%) [4, 9].

Large unilateral right-sided varicoceles can suggest the possibility of retroperitoneal or caval pathology, such as retroperitoneal tumors, and most clinicians would order further imaging to rule out an underlying pathology. Another contributing factor to the formation of varicoceles is incompetent (acquired or congenital) or absent (congenital) venous valves in the testicular veins that allow retrograde reflux of blood, which can cause venous dilation [10]. Rarely, varicoceles can result from the nutcracker syndrome as well, where the left renal vein is compressed between the superior mesenteric artery and the aorta, producing high pressure in the left testicular vein [11].

Pathophysiology

While varicocele formation is usually the result of anatomic or functional inadequacy of the drainage system, there is ongoing debate about the mechanism of spermatogenic impairment in the setting of a varicocele. The leading theory postulates that poor venous drainage disrupts the countercurrent exchange of heat from the spermatic cord causing a relative hyperthermia of the scrotum that affects both testes [12]. The cellular processes of the testis are exquisitely sensitive to increased temperature, and hyperthermia causes reductions in testosterone synthesis by Leydig cells, injury to germinal cell membranes, altered protein metabolism, and reduced Sertoli cell function [13]. While there has been conflicting reports on varicocele's effect on testosterone production, recent studies have shown that varicocelectomy alone may result in improved testosterone levels in men with low testosterone [14, 15]. When a varicocele is ligated, scrotal temperature is reduced to normal [16].

The impaired venous drainage can also cause hypoxia, poor clearance of gonadotoxins, and elevated levels of oxidative stress. The level of oxidative stress correlates with the varicocele grade [17], and treatment of the varicocele decreases the level of reactive oxygen species, although not to the levels of fertile men [18]. Other theories include decreased perfusion due to an increased level of catecholamines (specifically norepinephrine) in refluxing venous blood. The elevated level of norepinephrine causes constriction of the intratesticular arterioles and decreased arterial perfusion [19]. Prostaglandins have also been found to be elevated in the testicular vein of infertile varicocele patients compared to fertile patients with varicoceles, but the clinical significance of this is not known [20].

Diagnosis

While there are excellent imaging studies that can assist in the diagnosis of varicoceles, the most clinically relevant diagnostic tool remains the physical examination. It is important that any examination for the diagnosis of varicoceles

includes a scrotal examination while the patient is standing as well as supine. Right-sided varicoceles or varicoceles that fail to decompress while supine suggest the presence of other pathology such as renal tumors, retroperitoneal masses, and rarely, the presence of sinus inversus. Classically, a severity grade is assigned to the varicocele based on physical examination findings:

- **Subclinical**: Not palpable or visible at rest or during Valsalva maneuver but seen on scrotal ultrasound.
- **Grade I (small)**: palpable only during the Valsalva maneuver.
- **Grade II (moderate)**: palpated without Valsalva.
- **Grade III (large)**: visible through the scrotal skin and classically described as feeling like a "bag of worms."

This unvalidated grading scheme has made outcome research difficult. Hargreave and Liakatas compared the examination findings between 2 experienced physicians and found grade discordance in 26% of patients.

Due to this variability in physical examination findings and given the fact that most believe varicoceles to be the most common reversible cause of male infertility, physicians have developed imaging modalities to assist with diagnosis, though none are as important or clinically relevant as the physical examination.

Ultrasound

Scrotal ultrasound has the advantage of being noninvasive as well as free from ionizing radiation. It can diagnose other scrotal pathology and provide more accurate testicular size measurements. Ultrasound can detect non-palpable varicoceles with a >94% sensitivity and specificity [21]. Because repair of these "subclinical" varicoceles are not thought to improve fertility, routine ultrasound use for varicocele screening is not recommended nor is the treatment of subclinical varicoceles. Its use may be required in special cases such as large body habitus or equivocal physical examination findings. Varicoceles are diagnosed on ultrasound with the demonstration of reversal of venous blood flow with Valsalva or spermatic vein diameter >3 mm [22].

Venography

Venography of the testicular veins can be used for both diagnosis and treatment of varicoceles. Venography is the most sensitive imaging modality, with nearly 100% sensitivity (i.e., almost all patients with clinical varicoceles will demonstrate reflux on venography). Unfortunately, its clinical utility is limited since up to 70% of patients without a clinical varicocele are found to have reflux during venography [23, 24]. This modality is also used to access the testicular veins for percutaneous

treatments such as sclerotherapy and embolization, which will be discussed later. While not indicated for routine screening or characterization of varicoceles, it can be used to help delineate post-treatment recurrences and then embolize the remaining veins.

Other Diagnostic Modalities

Other techniques such as magnetic resonance imaging (MRI), thermography (measuring the temperature of the scrotum), and radionucleotide scans using technetium 99 m pyrophosphate have been used to diagnose varicoceles. These techniques have no better outcomes than the modalities discussed previously and are limited by their cost, inferior diagnostic accuracy, or both. They should not be routinely used.

Semen Characteristics

In the original World Health Organization (WHO) study, the 9034 men with infertility demonstrated lower sperm concentration and motility compared to their fertile peers [4]. Since that time, the WHO reference ranges and evaluation methods for semen analysis have changed 3 times (1992, 1999, 2010). Using the most recent WHO criteria, Agarwal et al. performed a meta-analysis of 10 studies that showed the semen of men with varicoceles has a reduced sperm count, decreased motility, and abnormal morphology. The semen volume remained within normal limits [25].

These findings in addition to an increased number of tapered forms and immature cells make up the originally described "stress pattern" [26]. Unfortunately, this characteristic stress pattern is not a sensitive marker for varicocele as it is seen in other pathology and should not be used to diagnose a varicocele alone.

Treatment

Indications

The American Society for Reproductive Medicine recommends that a varicocele be treated when all or most of the following criteria are met [27]:

1. The varicocele is palpable on physical examination of the scrotum.
2. The couple has known infertility or the male desires future fertility.

3. The female partner has normal fertility or a potentially treatable cause of infertility, and time to conception is not a concern.
4. The male partner has abnormal semen parameters.

In these patients, a successful repair may halt further testicular damage [6, 28], improve spermatogenesis [29, 30], and improve Leydig cell function and testosterone production [14].

Men with pain that is thought to be attributed to a varicocele are also candidates for varicocele repair as up to 80% may experience symptomatic improvement with treatment [31]. Patients with subclinical varicoceles should not be treated due to a lack of efficacy [32].

The decision to treat varicoceles in the adolescent does not follow the same rules set out by the American Society for Reproductive Medicine since most adolescents are not planning on conceiving a child. In this patient population, the goal of therapy is to halt testicular atrophy and preserve function for future conception. Diagnosis in this heterogeneous group is difficult due to the rapidly changing testicular size and hormonal milieu as well as the different stages of development that happen at different ages for different patients. Some authors have recommended treating a palpable varicocele if there is a 2 ml or 20% change in testicular volume compared to the unaffected testis [33]. This is supported by a small study that found recovery of testicular volume or so-called catch-up growth in 80% of adolescents after repair of grade 2 or 3 varicoceles [28]. A second study showed that 43.8% of patients actually have hypertrophy of the left testis following repair of the ipsilateral varicocele. A more recent study has put these results in question though. Kolon et al. reviewed 71 boys being evaluated for varicoceles with ipsilateral testicular hypotrophy who received no surgical repair [34]. They found that 85% had a volume differential of less than 15% after two years. However, there could be a selection bias as a large subset of their original cohort underwent surgical repair of their varicoceles. These results can be difficult to apply to clinical practice due to variations in measurement techniques (orchidometer, calipers, ultrasound) and accuracy [35]. A pretreatment semen analysis can help to determine when to intervene in an adolescent varicocele, though this can be challenging to obtain in this population. Furthermore, there are no established normal ranges for semen analysis in this age-group. Given the controversy surrounding the adolescent varicocele, a shared decision-making approach must be taken with the family to determine whether surgery is right for their child.

In the absence of testicular asymmetry, adolescents with varicoceles should be followed with annual testicular size assessments (preferably ultrasound, given its higher accuracy), as well as semen analysis if possible to assist in determining which patients may benefit from varicocele repair.

Percutaneous Treatment

The first percutaneous treatment of a varicocele was described in 1978 when Lima and colleagues used hypertonic glucose and ethanolamine oleate to sclerose the testicular vein [36]. This was followed by the use of detachable balloons [37] and coils [38] to occlude the vein. Complications using this technique include increased radiation exposure, balloon migration [39], and injury to the femoral vein while obtaining access. A variation of the technique, antegrade scrotal sclerotherapy [40], has been described with high success rates, though larger studies with longer follow-up are needed [41].

Due to recurrence rates as high as 11% [42] and lower initial success rates, percutaneous embolization is best used for persistent or recurrent varicocele after surgical repair [13, 43].

Surgical Repair

Tulloch performed the first-reported varicocele ligations for infertility in 1955 [44]. Since then, it has become the most commonly performed procedure for the correction of male factor infertility [45]. There are many techniques for varicocele repair, each with their pros and cons regarding success rates, recurrence, hydrocele formation, and other postoperative complications. In this chapter, we will discuss the retroperitoneal, laparoscopic, inguinal, and subinguinal approaches. The latter 2 are ideally performed with an operating microscope.

The retroperitoneal approach, also known as the Palomo procedure, is performed by making an incision at the external inguinal ring, splitting the external and internal oblique muscles, and exposing the spermatic cord vessels near the ureter. This technique gives the surgeon the ability to isolate the veins more proximally where there are usually only 1 or 2 large veins that need to be ligated as opposed to the inguinal approaches where there are usually many more veins that must be ligated to have a successful outcome. The biggest disadvantage of this technique, however, is the higher recurrence rate, generally around 15% [46–48], especially when preserving the artery, as failure is usually from preservation of the fine venae comitantes of the artery. Dilated cremasteric veins are also not identified during this approach and may be a source of recurrence [49]. Less commonly, parallel collaterals can exit the testis and bypass the ligated veins [50, 51]. A microscopic retroperitoneal varicocelectomy has been recently described in a small study with similar outcomes to the microscopic subinguinal approach [52].

The laparoscopic varicocelectomy is essentially a retroperitoneal approach performed through the abdomen and with laparoscopic instruments. The magnification provided by the laparoscope allows better visualization of the testicular artery, and with experience, the lymphatics may be visualized and preserved as well to decrease the rate of postoperative hydrocele formation [53]. As this is the only

intra-abdominal approach for the treatment of varicoceles, this procedure has increased risk of serious complications such as injury to the great vessels or bowel during insufflation and port placement as well as other laparoscopic complications such as CO_2 air embolus. It also requires a general anesthetic and is usually more expensive. A few centers are using single-port laparoscopic surgery to perform this procedure [54]. Recurrence rates are typically low (2% [55]) but can be as high as 17% [56]. According to a survey of the Pediatric Health Information System, a comparative pediatric database of 45 tertiary care pediatric hospitals, the laparoscopic approach is still the most commonly used in adolescents, likely due to the pediatric urologists familiarity with laparoscopic orchiopexy [57]. While the procedures discussed below have become gold standard for varicocele repair, this technique may be beneficial for the treatment of bilateral varicoceles [58].

The inguinal approach is the same used for a radical orchiectomy or hernia repair and is familiar to most urologic surgeons. An incision is made over the inguinal canal, dissection is carried down to the external oblique aponeurosis, which is opened, and the spermatic cord is encircled and delivered. The cord is then dissected, and all the internal spermatic veins are ligated, while the vas (and its vessels), testicular artery, and lymphatics are preserved. There appears to be a 3–15% risk of hydrocele formation when the operating microscope is not used [59]. Though the subinguinal approach has largely replaced the inguinal approach in most cases, there are some relative indications for the former. Men with solitary testis may benefit from this approach to minimize injury to the testicular artery since the artery should be larger and less branched more proximally. In children and prepubertal adolescents, it is recommended to open the external oblique fascia to facilitate identifying the artery as it is quite small in this age-group and can be difficult to find with the subinguinal technique. Other anatomic considerations that may favor inguinal over subinguinal approaches include a low or tight external ring and a short cord that cannot be brought out of the wound easily.

The subinguinal technique was first described by Marmar and colleagues in 1985 as a way to prevent opening the external oblique fascia and reduce postoperative pain [60]. While it had been used intermittently before 1992, Goldstein popularized the use of the operating microscope [61] when performing varicocele repairs, which greatly increased the ability to see and spare the artery and lymphatics [62]. In this approach, an incision is made below the external ring and the cord is located, grasped, and brought to the surface of the incision. A Penrose drain or a Fuchs platform (sterile tongue depressor slid through a Penrose drain) can be placed below the cord to hold it just above skin level to allow the surgeon to operate with both hands. The cord is dissected, and a micro-Doppler is used to identify arterial pulsations. Clips or suture ligatures are used to divide aberrant veins. This procedure is especially useful in patients with prior inguinal surgery (where the cord is likely scarred to the underside of the external oblique fascia), obese patients, high external rings, and patients with long cords. In both the inguinal and subinguinal approach, the testis can be delivered if necessary to visualize all possible avenues of venous drainage.

Complications

As mentioned earlier, hydrocele formation postoperatively from ligation of lymphatics can be a common complication, especially in the inguinal and laparoscopic approaches. This is concerning for infertile men as there is some data that a hydrocele can cause similar hyperthermia of the testis that a varicocele can [63]. With the advent of the microscopic subinguinal technique, hydrocele formation is very rare.

The testicular artery supplies two-thirds of the testicular blood supply [8], and injury to this artery can cause atrophy of the testis. In early renal transplant literature, where the entire cord, with the exception of the vas, was intentionally ligated to facilitate exposure of the iliac fossa, 14% of patients developed testicular atrophy and 70% had hydrocele formation [64]. Unfortunately, the true incidence of artery injury is not known, but has likely improved since the operating microscope began being used for varicocele repair [65]. In prepubertal boys, testicular atrophy is likely less common due to the potential for revascularization of the testis from the vasal and cremasteric arteries, evidenced by similar atrophy rates in both artery sparing and Fowler–Stephens orchidopexies performed for undescended testis [66].

Recurrence rates vary wildly in the literature from 0.6 to 45% [62, 67, 68]. A recent, albeit small, randomized controlled trial demonstrated a recurrence rate of 18% in the laparoscopic group, 13% in the open inguinal group, and 2% in the microscopic subinguinal group [69]. A meta-analysis of 4 randomized controlled trials also showed a statistically significant decrease in hydrocele and recurrent varicocele formation with the microscopic subinguinal technique compared to the laparoscopic and inguinal approaches [70].

Semen Analysis Outcomes

The outcomes of varicocele repair are the topic of much debate due to variable diagnostic criteria, follow-up, and outcomes reporting and the retrospective nature of most of the studies performed. A Cochrane review, updated for 2012, looked at 10 randomized controlled studies comparing varicocele repair with no treatment and found an odds ratio for pregnancy of 1.47 with a 95% confidence interval of 1.05–2.05 [3]. Given this confidence interval and what they deemed as low quality of evidence, the authors concluded that the treatment of varicoceles in subfertile men "may improve a couple's chance of pregnancy," but the "findings are inconclusive" [3]. This meta-analysis has been criticized for including studies in which the majority of patients had normal semen analysis and subclinical varicoceles; many more studies support varicocele repair improving semen parameters and pregnancy rates [71]. Multiple studies have shown an improvement in semen parameters after varicocelectomy in infertile men with clinical varicoceles. A meta-analysis of 17 studies by Agarwal et al. found post-varicocele repair semen

analysis had a mean increase in sperm density of 9.7 million/mL, motility increase of 9.9%, and WHO sperm morphology improvement of 3% [1]. A meta-analysis of adolescents did not show a difference in post-varicocele repair semen analysis, but did show a significant improvement in bilateral testis size (2.9 ml increase on the affected side and 1.5 ml increase on the contralateral side) [72]. Larger varicoceles seem to exert a greater negative effect on semen parameters than smaller ones and also show a more substantial improvement after varicocele repair. Steckel et al. found that the fertility index (sperm count × motility %) of men with grade 3 varicoceles improved to a greater degree (128%) than men with grade 1 (27%) or grade 2 (21%) varicocele [73]. Similarly, repair of bilateral varicoceles also produced an increased benefit compared to the repair of a unilateral varicocele [74]. Other studies have shown improvement in the sperm penetration assay [75] and a decrease in DNA fragmentation [76] and oxidative stress levels [77].

Outcomes in Azoospermic Men

For men with azoospermia, even a modest improvement in semen quality after varicocele ligation can significantly impact a couple's fertility options. Multiple studies have shown that a number of azoospermic men can have unassisted, natural pregnancies after clinically significant varicocele repair [78, 79]. In the first large series reported, Matthews et al. evaluated 22 men with azoospermia and 56 men with oligoasthenospermia (OAT) [80]. After microscopic varicocelectomy, 55% of the azoospermic men had motile sperm with an average motile sperm count of $2.2 \pm 1.1 \times 10^6$. Fourteen percent of these men had a pregnancy during follow-up, including 2 patients (9%) who were able to conceive naturally [80]. Gat et al. examined a prospective cohort of 101 men with severe OAT, 32 of whom had true azoospermia [81]. These men were all treated with embolization of their varicoceles. Of the overall group, 82% showed clinically and statistically significant improvement in their semen parameters, while 56.2% of the azoospermic men had improvement. There were 9 pregnancies (26%) in the azoospermic group, 4 (12%) unassisted, and 5 (15%) by intracytoplasmic sperm injection (ICSI) (1 was a twin pregnancy), all resulting in live births [81].

Esteves and Glina looked at varicocelectomy outcomes in azoospermic men based on testicular histology [82]. In their study, 17 men underwent bilateral testis biopsy followed by varicocele repair of clinically significant varicoceles. Histology revealed hypospermatogenesis in 6 men, maturation arrest in 5, and Sertoli cell only in 6. Only men with hypospermatogenesis (5/6) or maturation arrest (3/5) had improvement after varicocele repair. One patient with hypospermatogenesis (12.5%) contributed to an unassisted pregnancy. Successful testicular sperm retrieval for ICSI was achieved in 44.4% individuals who did not improve after surgery [82]. In a similar study, spermatozoa were detected in the ejaculate in 18 of 30 (60%) of men with hypospermatogenesis; 12 of 26 (46%) of those with maturation arrest; and one of 34 (3%) with Sertoli cell only after varicocele repair [83].

Based on these data, some urologists recommend a testicular biopsy in men with non-obstructive azoospermia before varicocelectomy.

Treatment of varicocele may reverse testicular dysfunction and improve spermatogenesis, even in patients with azoospermia. In carefully selected patients (possible with testicular biopsy), varicocele repair may spare some azoospermic patients the need for testicular sperm extraction as preparation for in vitro fertilization (IVF)/ICSI and offer the possibility of natural conception. Since achievement of pregnancy after IVF is higher when spermatogenesis is better, even patients who are unable to conceive naturally may benefit from the treatment of their varicoceles before IVF.

Testosterone

In men with low testosterone, varicocele repair alone can result in improved testosterone levels. In a study of 53 patients who underwent varicocelectomy for infertility, mean serum testosterone increased from 319 to 409 ng/dL [14]. The authors concluded that while improvement in serum testosterone does not necessarily cause a direct improvement in semen quality, varicocelectomy may improve hormonal and spermatogenic function. A similar study found a statistically significant increase in mean testosterone of 109.1 ng/dL in 83% of the patients after undergoing varicocele repair [84]. There seemed to be no correlation between grade of varicocele and increase in testosterone postoperatively.

Pregnancy Rates

Most of the larger published retrospective studies found a 30–50% pregnancy rate after varicocele repair [85–87]. Pregnancy usually occurred an average of 8 months after varicocelectomy [88]. A meta-analysis of 5 studies from 1988 to 2002 comparing varicocelectomy versus no treatment found the pregnancy odds ratio to be 2.87 in favor of the treatment group. The number needed to treat was 5.7. The overall pregnancy rate varied significantly between studies though, from 20 to 60% [89]. In the largest series to date, Goldstein's group has found a 43% pregnancy rate at 1 year and a 69% rate at 2 years when female factor infertility was excluded [90]. A well-designed prospective randomized trial by Madgar et al. randomized 45 men to retroperitoneal varicocele ligation or no treatment [91]. Pregnancy rates were 6 times higher in the intervention group compared to the control group (10%). Even more convincingly, the control group then underwent varicocelectomy after 1 year in the study and their pregnancy rates increased by fourfold post-procedure. The most recent prospective, randomized controlled trial comparing pregnancy rates in patients who underwent microscopic subinguinal varicocelectomy versus no

treatment was able to enroll 145 patients who were equally divided between each group [92]. Spontaneous pregnancy was achieved in 32.9% in the varicocelectomy group versus only 13.9% in the control group within the first year.

Varicocele Repair and Assisted Reproductive Technology

Cayan and colleagues studied a cohort of 540 couples to determine whether varicocele repair would change patient candidacy for assisted reproductive technologies (ARTs) [93]. They divided the patients into 4 groups based on their total motile sperm count: ICSI candidates (<1.5 million), IVF candidates (1.5–5 million), intrauterine insemination (IUI) candidates (5–20 million), and natural birth candidates (>20 million). All patients underwent either a bilateral (73%) or unilateral (27%) microscopic varicocelectomy. Postoperatively, half of the patients had a >50% increase in their total motile sperm count; 31% of couples moved from the ICSI and IVF groups to the IUI and natural birth groups; and 42% of IUI candidates were upgraded to natural birth candidates.

The sperm cutoffs for groups of patients in this study were somewhat arbitrary, and some centers can perform ART with even less motile sperm than in the groupings that Cayan and his colleagues set. Also while the overall natural birth rate was 36.6%, there is no data on pregnancy rates using ART in the other groups. Nevertheless, this study and others have shown that performing varicocele repairs, even in azoospermic men or those couples that may still need ART, can decrease their likelihood of needing the most aggressive and expensive forms [80, 94–96].

Varicocele repair is also much more cost-effective than ART. A cost analysis of varicocelectomy versus primary IVF/ICSI found that the cost per delivery with ICSI was $89,091, while the cost per delivery for varicocelectomy was only $26,268 [97]. Even assuming the highest published ICSI success rates at the time, varicocele repair was still over more than 2 times less costly. Penson et al. looked at the cost-effectiveness per live birth of 4 different treatment algorithms: observation, varicocelectomy followed by IVF (if unsuccessful), IUI followed by IVF (if unsuccessful), and immediate IVF [98]. Each additional live birth that resulted from choosing varicocelectomy/IVF over observation costs $52,152, while each additional birth that occurred by electing IUI/IVF over varicocelectomy/IVF costs $561,423.

In properly selected men, varicocele repair is more cost-effective and successful than ART, but it can also improve results when ART is ultimately needed. In couples undergoing IUI for male factor infertility, Daitch and colleagues found improved success after varicocelectomy [99]. In their study of 58 couples, post-wash total motile sperm count was no different in the patients who underwent varicocelectomy versus those who did not, but both pregnancy rates (11.8% vs. 6.3%) and live birth rates (11.8% vs. 1.6%) favored the varicocele repair group, indicating that varicocele treatment may improve pregnancy outcomes even if semen analysis does not change.

Conclusion

Treatment of clinically significant varicoceles results in significantly improved semen parameters in 60–80% of men and pregnancy rates of 20–60%, especially when a subinguinal microscopic varicocele ligation is performed. Overall, 30–50% of couples who were felt to require ART due to low semen quality may be able to avoid this after varicocele treatment, while also being more cost-effective than ART alone. Varicocelectomy can increase the chances of successful conception when used in conjunction with ART.

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Chapter 13
Treating Erectile Dysfunctions

Ahmad Majzoub, Haitham El Bardisi and Mohamed Arafa

Introduction

Erectile dysfunction (ED) is defined as the persistent inability to attain or maintain penile erection sufficient for satisfactory sexual performance [1]. The 1992 National Institutes of Health (NIH) Consensus Development Conference replaced the word "impotence" with ED, a more precise term that is independent from sexual desire, orgasm, and ejaculation [1]. Disorders of penile erection have long been considered to be of psychogenic or of age-related physiological origin. This belief was reformed after an enhanced understanding of the physiology and pathophysiology of penile erection made possible with newer diagnostic modalities evolving in the 1980s and 1990s. With an increasing knowledge about possible disturbances of penile erection, new organically oriented therapeutic options have been developed, which have revolutionized the treatment of ED. Several studies have been carried out worldwide to establish the prevalence of ED. The Massachusetts Male Aging Study is the first, community-based study to report a 52% overall prevalence of ED in men 40–70 years of age [2]. In 1995, an estimated 152 million men worldwide were affected by ED, with projections that this number could increase to 322

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million by 2025 [3]. Despite its benign nature, ED may affect the physical and psychosocial well-being of sufferers, causing a significant impact on their quality of life (QoL). Recent evidence has pondered ED as an early manifestation of cardiovascular disease (CVD) suggesting that its occurrence should not be dealt with as only a QoL issue but also as a potential warning sign of a more serious medical condition [4, 5].

Erectile Physiology and Pathophysiology

An understanding of normal erectile function is necessary to help understand the different pathophysiologic mechanisms and/or treatment options involved in ED. Erection is the consequence of a complex interaction between neurochemical and vascular smooth muscle responses to physiologic or erotic stimuli. Three types of erection exist: psychogenic, reflexogenic, and nocturnal. The brain plays a pivotal role in sexual arousal involved in psychogenic erection. Influenced by visual, sensory, or cognitive stimuli, several brain centers interact to initiate a sexual response. Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have allowed a greater understanding of the contribution of different areas in the brain. Areas in the frontal and temporal cortex as well as the medial preoptic and paraventricular nuclei demonstrated significant activation during sexual excitement [6]. The autonomic nervous system conducts the sexual response through direct stimulation of erectile tissue. Influenced by signals from higher brain centers, the parasympathetic nervous system originating from the sacral spinal cord (S2–S4) is responsible for erection. Parasympathetic nerve fibers travel through the pelvic plexus where they are carried by the cavernosal branches innervating the penile tissue. The sympathetic nervous system, on the other hand, originates from thoracic vertebra T10-L2 and travels through the superior hypogastric nerve into the pelvic plexus and cavernosal nerves to instigate penile detumescence.

Reflexogenic erections are the result of tactile penile stimulation and are essential in maintaining erection during sexual activity. After reaching the spinal cord, impulses delivered through the sensory somatic nerves, either ascend to the brain to stimulate sensory perception or activate the parasympathetic autonomic nuclei to induce erection. This type of erection is preserved in patients with upper spinal cord injury. Finally, nocturnal erection occurs mainly during rapid eye movement (REM) sleep. It is believed to be secondary to activation of the cholinergic lateral pontine neurons and suppression of the adrenergic and serotonergic neurons of the locus ceruleus and midbrain, as confirmed by PET scanning of humans during REM sleep [7].

Anatomically, the penis is a highly vascular organ composed of two cylinders of erectile smooth muscle tissue that are capable of withstanding the eightfold increase in penile blood flow during erection [8] (Fig. 13.1). A tough corporal covering called *tunica albuginea* exists and is principle in the physiology of erection.

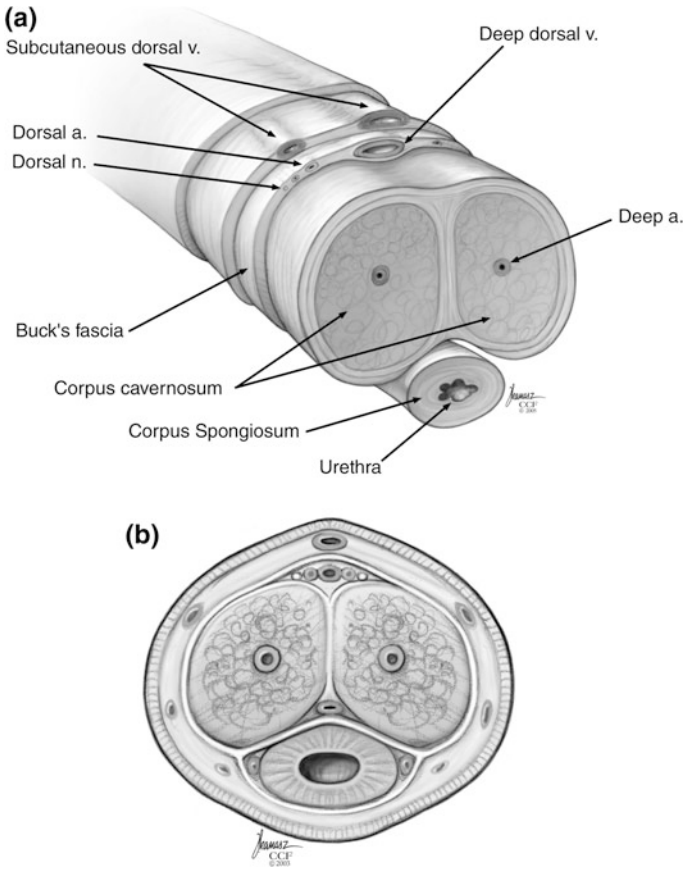

Fig. 13.1 **a**, **b** Anatomy of the penis. © CCF 2003, 2005. Reprinted with permission

Smooth muscle relaxation is triggered by the nitric oxide/cyclic guanosine monophosphate (NO/cGMP) system. In response to sexual stimulation, NO produced by nitric oxide synthase (NOS) is released by end-neurons and endothelial cells in the cavernosal tissue stimulating the conversion of guanosine triphosphate (GTP) into cGMP. Consequently, cGMP interacts with protein kinase G causing an increase in intracellular calcium and resulting in smooth muscle relaxation. As blood flows into the penis, a buildup in pressure occurs secondary to the effect of the tunica albuginea in holding penile stretch. This mechanically compresses the subtunical venules responsible for venous return causing further increase in penile pressure or maximal erection. Detumescence results from the inactivation of the NO/cGMP pathway. Phosphodiesterases (PDEs), specifically PDE5, catalyzes cGMP causing its inactivation. An understanding of this chemical activity formed the basis for using PDE5 inhibitors in the management of ED.

Table 13.1 Classification of male ED

Organic	Psychogenic
Vascular	Generalized
Arterial	Absence/decline in sexual reusability
Venous	Chronic disorder of sexual intimacy
Mixed	Situational
Neurogenic	Partner-related
Endocrinologic	Performance-related
	Psychological distress- or adjustment-related

The International Society of Impotence Research has classified ED broadly into organic and psychogenic ED [9] (Table 13.1). Organic ED can be the result of vascular, neurologic, or endocrinologic ailments. Arterial insufficiency and/or veno-occlusive dysfunction are the major components of vascular ED. Common risk factors associated with arterial insufficiency include hypertension, dyslipidemia, smoking, diabetes mellitus (DM), perineal or pelvic trauma, and pelvic irradiation [10]. The overlap in risk factors between ED and CVD, together with a higher incidence of elsewhere arterial lesions in impotent men than the general population, has led researchers to consider ED to be a manifestation of generalized or focal arterial disease [11]. Failure of adequate venous occlusion can be seen in patients with Peyronie's disease, DM, or in patients with a traumatic history to the tunica albuginea such as penile fracture.

A neurologic origin is encountered in about 10–19% of ED cases [12]. It commonly results from iatrogenic nerve injury that occurs with pelvic and retroperitoneal surgeries. Other traumatic and medical conditions such as pelvic fractures, spinal cord injuries, Parkinson's disease, stroke, spina bifida, disc herniation, syringomyelia, transverse myelitis, and multiple sclerosis are also associated with neurogenic ED. Hypogonadism is the most recognized endocrine condition related to ED. Testosterone increases sexual desire, frequency of sexual acts, and occurrence of nocturnal erections [13]. Despite this, treatment of hypogonadal impotent men with exogenous testosterone reportedly improved their libido with little effect on potency [14]. Previously, about 90% of impotent men were thought to suffer from psychogenic ED [15]. Such belief has been replaced by an understanding that ED originates from either functional or physical circumstances. Psychogenic ED may be from an exaggerated sympathetic outflow as few clinical studies have demonstrated higher levels of serum norepinephrine in patients with psychogenic ED than in normal controls or patients with vascular ED [16].

Case Scenario

A 47-year-old gentleman presents to a men's health clinic complaining of erectile dysfunction that he has been noticing over the past 3 months. He reports weak erections, early detumescence, and much lower frequency of weaker morning

erections. He was diagnosed with DM 4 years earlier and is on oral hypoglycemic agents with suboptimal control in his blood sugar as evidenced from a recent HbA1c of 8.3%. He also has had hypertension for 8 years. Five months ago, his primary care physician added a beta-blocker (atenolol) to his antihypertensive regimen. He denies any issues with ejaculation or with sexual desire. He is a non-smoker. On physical examination, his body mass index (BMI) is 27 kg/m^2. He has normal secondary sexual characteristics. His abdomen is soft and lax with no palpable masses and intact hernia orifices. His genital examination reveals a normal penis and urethral orifice with no palpable penile plaques. Both testes are of normal size and consistency. No other scrotal abnormalities have been detected. Peripheral pulses are symmetrically palpable.

Initial evaluation of a patient complaining of ED should entail a sexual, medical, and psychosocial history together with a focused physical examination of the abdomen, genitalia, and lower extremity pulsations [1]. Details such as onset, duration, progression, and sustainability of symptoms with different sexual stimulants are very important. Additionally, the frequency and strength of morning erections and the patient's ability to ejaculate during sexual encounters would help the clinician differentiate an organic from a psychogenic origin of symptoms (Table 13.2). The nature of symptoms has to be explored such as whether the patient experiences weak erections from the start, or whether he is not able to maintain an erection. Such info would help differentiate between an arterial or venous etiology. Presence or absence of reported penile pain or deviation during intercourse would suggest the presence of Peyronie's disease. Hypogonadism is also suspected when symptoms of decreased sexual desire, generalized fatigability, sleepiness, and lack of concentration exist. The patient's past medical, surgical, medication, social, and habitual history should be collected in detail searching for risk factors for the development of ED. This permits an understanding of the circumstances leading to ED but more importantly allows for the identification of modifiable risk factors, such as the use of medications known to alter erection (as in our clinical scenario). A complete list of risk factors is presented in Table 13.3.

Aiming to collect more objective information, a number of questionnaires have been developed to assist clinicians in their evaluation of patients. The most

Table 13.2 Factors differentiating between organic and pyscogenic ED

	Organic ED	Psychogenic ED
Onset	Gradual	Sudden
Duration	Long	Short
Progression	Progressive	Intermittent
Reaction to different sexual stimuli	Persistent	Inconsistent
Morning erections	Weak, less frequent	Normal
Ejaculation	Normal	Difficult/absent
Psychosocial problems	Secondary to ED	Long history
Anxiety	Secondary to ED	Primary

Table 13.3 Risk factors for erectile dysfunction

Risk factors for erectile dysfunction
Medical history:
Diabetes Mellitus
Hypertension
Coronary artery disease
Cerebrovascular disease
Obesity
Hypogonadism
Parkinson's disease
Multiple sclerosis
Pelvic/spinal cord injury
Surgical history:
Radical pelvic surgery (prostatectomy, cystectomy, low anterior resection)
Retroperitoneal surgery (lymph node dissection, aortic aneurysm, resections)
Orchiectomy
Medications/treatments:
Diuretics (Hydrochlorothiazide, Furosemide)
Antihypertensives (Propranolol, Atenolol, Metoprolol, labetalol, Enalapril, Captopril, Methyldopa, Verapamil, Nifedipine, Hydralazine)
Antihistamines (Dimenhydrinate, Diphenhydramine, Hydroxyzine, Meclizine, Promethazine)
Antidepressants (Fluoxetine, Sertraline, Amitriptyline, Clomipramine, Buspirone, Diazepam, Imipramine, Lorazepam)
Parkinson's disease drugs (Bromocriptine, Levodopa, Benztropine)
Antiarrhythmics (Disopyramide)
Muscle relaxants (Cyclobenzaprine, Orphenadrine)
Nonsteroidal anti-inflammatory drugs
Histamine H2-receptor antagonists (Cimetidine, Nizatidine, Ranitidine)
Chemotherapy medications (Busulfan, Cyclophosphamide)
Prostate cancer drugs (Flutamide, Leuprolide)
Pelvic and retroperitoneal radiotherapy
Habitual use of the following substances:
Amphetamines
Barbiturates
Cocaine
Marijuana
Methadone
Nicotine
Opiates

commonly used questionnaires include the International Index of Erectile Function (IIEF) and the Sexual Encounter Profile (SEP).

The IIEF is a 15-question tool that evaluates five domains of a sexual encounter: erectile function, orgasmic function, sexual desire, intercourse satisfaction, and global satisfaction [17]. It has been standardized and validated in several languages

[17] and is commonly used to monitor the degree of satisfaction to different therapeutic agents. A shorter version of the IIEF, termed the IIEF-5, has been developed as a more efficient sexual health inventory for men [18]. SEP, on the other hand, is a simpler method commonly used in clinical trials involving pharmacologic therapies for ED. It mimics a diary where men respond to a series of yes or no questions after each sexual attempt [19]. Specifically, the questions are as follows: Q1—Were you able to insert your penis into your partner's vagina? and Q2—Did your erection last long enough for you to have successful intercourse?

Physical examination is initiated with an assessment of body habitus and secondary sexual characteristics. Obesity is a well-known risk factor for ED and has been significantly linked to hypogonadism. Blood pressure and heart rate should be measured in every patient. Abdominal examination then follows in the standard manner with special attention on scars of prior surgeries on inspection, presence of masses or hernias on palpation, and an audible bruit on auscultation. Penile anatomy is fully considered during genital examination. Circumcision status, urethral orifice location, and presence of penile plaques during palpation should be noted. If penile deviation is reported, the patient should be asked to take pictures of his erect penis to estimate the angle of deviation. Scrotal examination for assessment of testicular size and consistency and presence of other scrotal pathology is then performed. A rectal examination should be performed in every patient older than 40 years. Finally, lower extremity examination looking for lower limb edema and abnormal peripheral pulses is important as it would suggest cardiac and vascular disease.

Management of Patients with Erectile Dysfunction

Workup

Information gathered during the initial encounter should provide insight on the type and extent of laboratory and radiologic investigations to be ordered. The International Society for Sexual Medicine recommends a fasting glucose, lipid profile, and, in select cases, a hormone profile in patients presenting with ED [20]. Men reporting a hypoactive desire or who are known to have DM should be tested and potentially treated for low testosterone levels [21]. When low serum testosterone is detected initially, a complete hormonal evaluation is warranted to differentiate between primary and secondary hypogonadism. The third Princeton Consensus Conference on sexual dysfunction and cardiac risk recommends evaluation of cardiovascular risk in men with ED and no known CVD. The aim is to assess patients' exercise ability to ensure a cardiovascular health that is consistent with the physical demands of sexual activity before prescribing treatment for ED. Moreover, the consensus highlights the value of early detection of cardiovascular

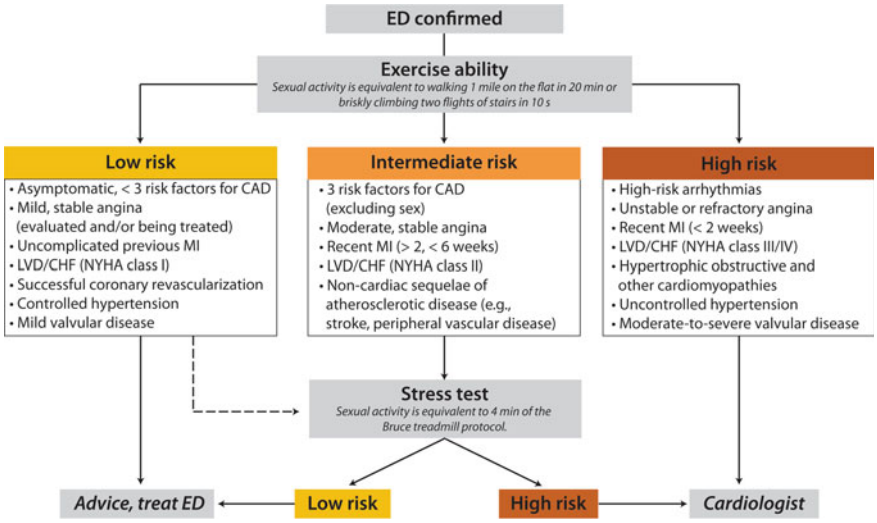

Fig. 13.2 Algorithm for evaluation of cardiovascular risk in patients with ED and no known history of CVD. *LVD* left ventricular dilatation, *CHF* congestive heart failure; *NYHA* New York Heart Association; *CAD* coronary artery disease; *MI* myocardial infarction

risk in an asymptomatic patient who would benefit from cardiovascular risk reduction [22] (Fig. 13.2).

Specialized Diagnostic Tests

The *intracavernous injection test* is seldom performed alone as it provides limited information about the penile vascular status. A rigid erectile response occurring about 10 min after an intracavernosal injection of a vasodilator substance and lasting for 30 min generally indicates a functional, but not necessarily normal, erection. It serves, however, as a prognostic tool that would advocate patients who will respond to an intracavernous injection program. Moreover, it helps in the assessment of the angle of penile curvature in patients with Peyronie's disease.

Penile duplex ultrasonography is indicated in patients who fail in oral therapy, or who have a history of penile or pelvic trauma, Peyronie's disease or other suspected vascular causes for their ED. During this procedure, the penis is assessed songraphically looking for echogenic areas that would represent penile fibrosis. Blood flow inside the corpora cavernosa is then measured before and after the instillation of a vasodilator, allowing the differentiation between arterial and venous insufficiency. A peak systolic velocity lower than 25 cm/s is a generally agreed upon value indicating arterial insufficiency. End-diastolic velocity serves as a reflection of venous outflow; venous leakage is ruled out when the velocity is <5 cm/s.

Nocturnal penile tumescence testing is less commonly performed to differentiate between organic and psychogenic ED. It involves placing several bands around the penis, which are connected to a device capable of sensing and measuring the change in compression pressure that occurs with erection. Inadequate or absent nocturnal erections suggest organic dysfunction, whereas a normal result indicates a high likelihood of a psychogenic etiology [23].

Angiography was earlier utilized in the evaluation of patients with ED. Currently, it is rarely performed and its role is limited to patients with traumatic vascular injuries resulting in ED who are candidates for vascular reconstruction [24].

Treatment

Management of ED is primarily aimed at identification and treatment of its etiologic factors rather than treating its symptoms alone. Modifiable risk factors such as lifestyle habits or medication use should be reversed, when possible, either before or at the same time as specific therapy is initiated. A systematized treatment strategy is instituted that depends on efficacy, safety, and invasiveness, as well as cost and patient preference. As such, a thorough discussion about the different available options is necessary.

First-Line Therapy

Oral Pharmacotherapy

PDE5 inhibitors (PDE5i) are the most common form of treatment used in patients with ED. Their concept is simple: inhibit the catalytic activity of PDE5 enzyme thereby maintaining the cGMP-mediated smooth muscle relaxation and increase in penile blood flow [25]. Several potent PDE5i exist, namely sildenafil, tadalafil, vardenafil, and avanafil.

Sildenafil

Launched in 1998, sildenafil was the first PDE5i available in the market. It is effective 30–60 min after administration, and its efficacy lasts for up to 12 h. Its absorption is prolonged with heavy, fatty meals and is hence recommended to be taken on an empty stomach. It is available in doses of 25, 50, and 100 mg, however, a starting dose of 50 mg is recommended, which can be modified according to the patient's response and side effects. The efficacy of sildenafil has been initially reported in a dose–response study where improved erections were detected in

56, 77, and 84% of patients with ED taking 25, 50, and 100 mg, respectively [26]. These results were later reproduced in several studies utilizing various patient sexual function questionnaires [27, 28].

The efficacy of sildenafil in almost every subgroup of patients with ED has been successfully established. Patients with diabetes reported a 66.6% improvement in erections compared to 28.6% of men taking placebo [29]. Furthermore, 71% of patients with concomitant CVD reported improved erections when taking Sildenafil compared with 24% taking placebo [30].

Tadalafil

Tadalafil was approved as a treatment alternative for ED in 2003. It has a 30-min onset of action while its efficacy is maintained for up to 36 h [31]. Its absorption is not affected by food intake. Tadalafil is available in 10 and 20 mg on-demand doses and 5 mg once-daily dose.

Again, dose–response premarketing studies confirmed the efficacy of tadalafil, where after 12 weeks of treatment, improved erections were reported by 67% of patients taking a 10 mg dose and 81% of patients taking a 20 mg dose in comparison with 35% of men receiving placebo [31]. Postmarketing studies confirmed and reproduced this efficacy with an improvement in scores of IIEF, SEP2, and SEP3 [32]. Recent interest in the chronic use of once-daily dose of tadalafil emerged after reports showing comparable efficacy to on-demand regimens were released. One randomized study (n = 145) has even reported a significantly higher IIEF score in patients receiving daily compared to on-demand tadalafil [33]. Another randomized double-blind clinical trial, using five and 10 mg/day tadalafil for 12 weeks (n = 268) have shown that daily dosing was well tolerated and significantly improved erectile function. These results make tadalafil 5 mg once-daily dose a reasonable alternative to men concerned about spontaneity in sexual activity. Furthermore, tadalafil 5 mg has been recently approved by the US Food and Drug Administration (FDA) as a treatment alternative for men with benign prostatic hyperplasia based on meaningful improvement in obstructive lower urinary tract symptoms [34–36].

Vardenafil

Vardenafil became commercially available in 2003. Its efficacy is initiated 30 min from administration, after which it remains effective for about 8 h. Like sildenafil, vardenafil has to be taken on an empty stomach as its absorption is reduced by heavy, fatty meals. 5, 10, and 20 mg doses have been approved for on-demand treatment of ED. Animal studies have reported a tenfold more potent efficacy in PDE5i inhibition for vardenafil in comparison with sildenafil [37, 38]; this, however, was not translated into clinical efficacy in human trials [39].

Of men with ED, 66, 76, and 80% reported an improvement in erectile function after the administration of 5, 10, and 20 mg vardenafil, respectively, compared with 30% of men receiving placebo [40]. Recently, an orodispersible (ODT) form of vardenafil has been released, offering a convenient alternative that can be taken irrespective of food intake and exhibiting better bioavailability over film-coated tablets [41]. The efficacy of vardenafil ODT did not seem to differ from the regular formulation as demonstrated by several randomized controlled clinical trials [41–44].

Avanafil

Avanafil, the more recent member of the PDE5i group, has been FDA approved since 2012. Its very fast onset of action compared with the other PDE5i proves advantageous. After ingestion, avanafil reaches its maximum concentration in about 30–45 min, providing the ability to engage in sexual activity within 15 min of consumption [45]. Available doses are 100 and 200 mg, taken irrespective of food intake. A meta-analysis of four randomized clinical trials, involving a total of 1381 patients revealed a greater efficacy to avanafil 100 mg over placebo for successful vaginal penetration and intercourse and a less likelihood of dropout due to an adverse event [46].

Side Effects and Safety Precautions

Adverse event profiles of PDE5i are generally similar and include headache, flushing, nasal congestion, dyspepsia, and myalgia, which result from capillary smooth muscle dilatation in other parts of the body. More formula-specific side effects stem from individual drug cross-reactivity with other phosphodiesterases. Eleven PDE isoenzymes have been identified in the human body. PDE6, located in the retina, plays an important role in the transfer of light into nerve impulses. Inhibition of this enzyme, seen principally with sildenafil and vardenafil causes disturbances in color perception [26]. Tadalafil, on the other hand, cross-reacts with PDE11, an enzyme expressed in the heart, liver, pituitary, and prostate with physiological functions that are still not clarified [47]. A comparison of pharmacologic properties and side effect profile between the different PDE5 inhibitors is depicted in Table 13.4.

Cardiovascular Safety

All clinical trials demonstrated a fairly safe impact for PDE5i on cardiovascular health. No increase in myocardial infarction rates in patients receiving PDE5i has been reported. Moreover, exercise testing in men with stable angina receiving PDE5i did not show an adverse effect on total exercise time or time to ischemia [48, 49].

Table 13.4 Comparison of different PDE5 inhibitors

	Sildenafil (100 mg)	Tadalafil (20 mg)	Vardenafil (20 mg)	Avanafil (200 mg)
Pharmacokinetics				
T_{max} (h)	0.8–1	2	0.9	0.5
$T_{1/2}$ (h)	2.6–3.7	17.5	3.9	10.6
Duration (h)	0.5–4	1–36	0.5–5	6
C_{max} (μ[mu]g/L)	560	378	18.7	5161
AUC (μ[mu]g/h/L)	1685	8066	56.8	10867
Side effects				
Bioavailability	41	NA	15	NA
Headache	14	15	10	10.5
Flushing	12	3	11	4
Dyspepsia	7.1	15	3	1
Abnormal vision	4.3	–	<2	1–2
Myalgia	–	5	–	1.1

T_{max} time at which maximum serum concentration is achieved; $T_{1/2}$ *(half life)* time it takes a substance to lose half its biologic activity; *Duration* length of time the drug is effective; C_{max} maximum serum concentration; *AUC* area under the curve

Cardiac contractility and output as well as myocardial oxygen consumption were not influenced by either on-demand or chronic use of PDE5i.

Nitrate Use

Organic nitrates such as nitroglycerine and isosorbide mono/dinitrate, commonly used to treat angina can amplify cGMP accumulation resulting in life-threatening hypotension. As a result, they are considered an absolute contraindication for the use of PDE5 inhibitors. The duration of interaction depends on the pharmacokinetics of the PDE5i and nitrate used.

Nitroglycerine should be withheld for at least 24 h after the last dose of sildenafil or vardenafil and for at least 48 h after the last tadalafil dose in patients developing chest pain.

Alpha Blockers

Co-administration of PDE5 inhibitors and alpha blockers may result in a small additive effect on blood pressure reduction manifested as orthostatic hypotension. This effect appears to be more prominent within 4 h of alpha blocker treatment. Generally, PDE5i should be administered only after the patient has been stabilized on alpha blocker therapy. Such observations were more documented with less selective alpha blockers (doxazocin) compared to more selective drugs (tamsulosin) [50, 51].

Dosage Adjustment

PDE5 inhibitors are primarily metabolized by the cytochrome P450 enzyme CYP3A4. Therefore, drugs inhibiting the CYP3A4 pathway will reduce the metabolic breakdown consequently resulting in higher serum concentrations of PDE5i. Examples include antifungal agents such as ketoconazole and itraconazole, macrolide antibiotics such as erythromycin and clarithromycin, and human immunodeficiency virus (HIV) protease inhibitors such as ritonavir and saquinavir. Hence, the dose of the PDE5i should be reduced in patients receiving such medications. On the other hand, drugs inducing CYP3A4 activity, such as rifampin, phenobarbital, phenytoin, and carbamazepine, would enhance the breakdown of PDE5i, necessitating higher doses with such medications.

Clinicians prescribing PDE5 inhibitors are required to offer adequate counseling to ensure proper medication use. Errors in the methods of consumption of such medications are commonly encountered and are principally related to lack of sexual stimulation, inappropriate dosing, or timing and false beliefs. It is imperative to educate patients that sexual stimulation is mandatory for NO release and subsequently for the PDE5 inhibitors to function and without which no effect for the drug is expected. Moreover, an understanding of the onset, duration of action, and time to maximal concentration (Table 13.4) is required to guide patients on correct medication use. Patient education was found, in several uncontrolled studies, to help salvage an apparent non-responder to a PDE5i [52–54].

Vacuum Erection Devices (VED)

In 1874, John King was the first to utilize vacuum technology in the treatment of ED [55]. Such technology later underwent a series of adjustments until its FDA approval in 1982 [56]. Despite that the device initially faced strong skepticism among patients and physicians but was later appeased with reports documenting its safety and efficacy [57, 58]. A VED is comprised of three components: a cylinder, a battery- or manually operated vacuum pump, and constriction rings of varying sizes (Fig. 13.3). It works by passive engorgement of the corpora cavernosa caused by the negative pump pressure (100–225 mm Hg), which is followed by placement of constriction rings at the base of the penis to retain blood within the corpora. VED can be used in all ED etiologies with success rates that depend on proper instructions and practice [59]. It appears, however, that VED therapy may be more acceptable among elderly patients with occasional sexual intimacy, as younger patients may show limited acceptance because of its perceived “unnatural” erection [60]. Erections suitable for intercourse were reported by 90% of ED patients, who had satisfaction rates ranging between 27 and 94% [61]. The highest satisfaction rates were seen in men who had a motivated, interested, and understanding partner. Furthermore, Bosshardt et al. reported an improvement in nocturnal penile tumescence rigidity in VED users after 6 months of continued therapy [62].

Despite that, VEDs do not seem very attractive on the long run, as their use decreases to about 50–64% after about 2 years of therapy [63].

The commonest adverse events include pain, inability to ejaculate, petechiae, bruising, and numbness, which occur in <30% of patients [56]. Corporeal blood gas analysis studies revealed that ischemia could occur after 30 min of applying the constriction rings [62]. This resulted in recommending against ring application for >30 min to prevent ischemic injury to the penis. Serious adverse events (skin necrosis) can be avoided if patients remove the constriction ring within 30 min.

Contraindications to the use of VED are few and include patients with (1) a tendency for spontaneous priapism, such as patients with hemoglobinopathies; (2) severe penile anomalies (either congenital or acquired) [56]; and (3) bleeding disorders or on anticoagulation therapy due to higher risk of developing petechiae, ecchymosis, or hematoma [56]; although, such risk was not proven to exceed that of the general population [64, 65].

Low Intensity Shockwave Therapy (LISWT)

Shock waves are a form of acoustic waves capable of generating a pressure impulse. Its focus can be moderated, allowing energy concentration at a desired location [66]. As the focused waves interact with the targeted deep tissues, transient micromechanical forces are generated resulting in a number of biological changes. On vascular and smooth muscle tissue, LISWT is capable of stimulating angiogenesis and revascularization, making it an attractive modality to restore normal erectile function. Recently, LISWT has been proposed as a novel treatment for ED [66]. In the first randomized, double-blind, sham-controlled study, LISWT showed promising results in restoring the erectile function of men who are known responders to oral PDE5 inhibitors [67]. Moreover, patients with a more severe form of ED who are poor responders to PDE5i also demonstrated an improvement in penile hemodynamics and endothelial function, as well as erectile function [68]. The non-invasive rehabilitative nature of this treatment makes it an attractive new therapeutic option for men with ED. However, further studies are required before clear recommendations can be given.

Second-Line Therapy

Intracavernosal Injections

Beginning in the early 1980s after the reports of Virag [69] and Brindley [70], who demonstrated a rigid erection after the injection of vasoactive agents directly into

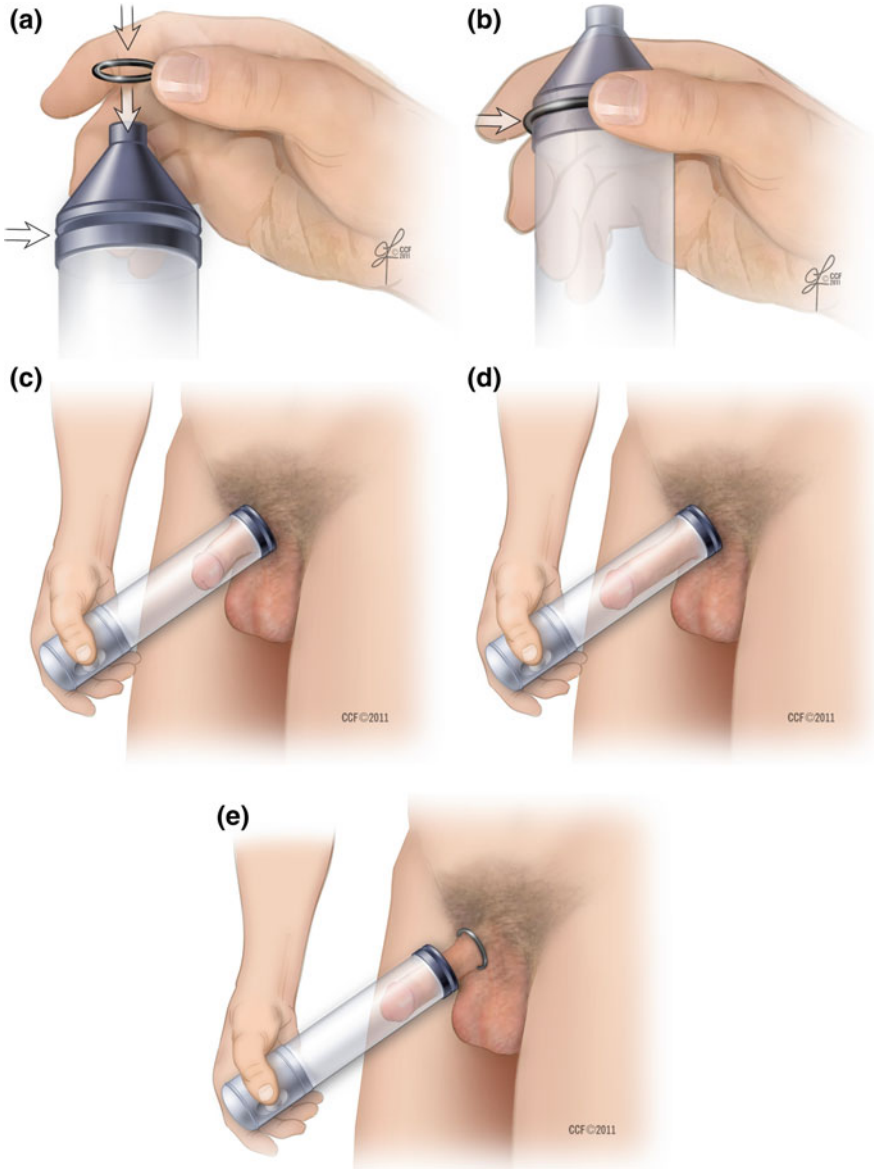

Fig. 13.3 Components and application of the vacuum erection device. **a** Vacuum pump and constriction ring. **b** Constriction ring on pump. **c** Vacuum pump placement. **d** Engorged penis. **e** Constriction ring placement on penis. © CCF 2011. Reprinted with permission

the corpora cavernosa, intracavernosal injections (ICI) became the first medical treatment modality for patients with ED (Fig. 13.4). Current practice guidelines recommend the use of ICI for patients not responding to oral PDE5i [1, 71].

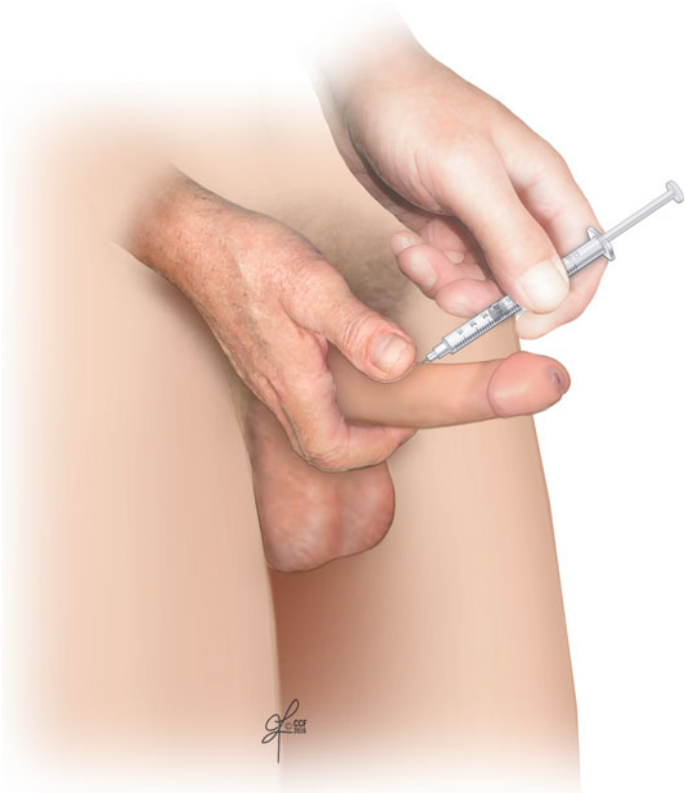

Fig. 13.4 Intracavernosal injection (ICI). © CCF 2016. Reprinted with permission

Several vasoactive agents either used as monotherapy or in combination therapy have been utilized to treat ED. Alprostadil (prostaglandin E1) is the first drug approved for intracavernosal injection [72]. It binds with PGE receptors, eliciting a cyclic adenosine monophosphate (cAMP) mediated smooth muscle relaxation response [73]. Almost 80% of alprostadil is metabolized by the lung accounting for the lack of significant cardiovascular side effects when administered intracavernosally [73]. A dose ranging from 10 to 40 μ(mu)g can be given, producing a variable dose-related response in rigidity and duration. Papaverine (20–80 mg) is another alternative known as a smooth muscle relaxant and vasodilator. It is often used in combination due to a higher incidence of side effects when used alone [71]. Phentolamine (0.5–1 μ[mu]g), on the other hand, is a non-selective α(alpha) blocker that causes smooth muscle relaxation [74]. It favorably used in combination due to an inferior efficacy when used as monotherapy [71]. Other compounds such as vasoactive intestinal peptide (VIP), linsidomine, or moxisylyte have been less commonly utilized.

Treatment is generally started with a lower dose of alprostadil and is gradually increased until desired response is achieved. If alprostadil alone does not produce a satisfactory response or when complications are reported with high doses, the addition of papaverine and or phentolamine is made.

In descending order, the reported side effects include the following: penile pain (37%), prolonged erections (4%), fibrosis (3%), hematoma (3%), ecchymosis (2%), and penile rash or edema (1%) [75]. Systemic side effects are uncommon and involve mild hypotension, especially when using higher doses. Contraindications for the use of ICI include hypersensitivity to any of the products, history of priapism, and history of bleeding disorders.

The efficacy rates of more than 70% for alprostadil monotherapy were reported by patients with ED [76]. Combination therapy, on the other hand, has the highest efficacy rates, reaching 92% [77]. Despite these favorable outcomes, ICI is associated with high dropout rates (41–68%) commonly in the first 2–3 months [78]. Reasons for discontinuation included desire for a permanent modality of therapy (29%), lack of a suitable partner (26%), poor response (23%), fear of needles (23%), fear of complications (22%), and lack of spontaneity (21%). Careful patient counseling is advisable prior to treatment to enhance patient satisfaction and minimize withdrawal [79].

Intraurethral Alprostadil

A less-invasive method of administration of alprostadil is through a medicated pellet (MUSE) inserted into the penile urethra (Fig. 13.5). It is usually given in doses between 500 and 1000 μ(mu)g for patients who do not wish to use ICI [80]. The physiologic response depends on the vascular interaction between the corpus spongiosum and the corpus cavernosum, which allows delivery of alprostadil into erectile tissue [80]. Success rates with intraurethral alprostadil are inferior to ICI (30–65.9%) [80–82].

The most common side effects include local pain (29–41%), dizziness and hypotension (1.9–14%), urethral bleeding (5%), and urinary tract infections (0.2%) [71].

Third-Line Therapy

Penile Prosthesis Implantation

Penile prosthesis implantation (PPI) is considered in patients not responding to pharmacotherapy or who request a more permanent solution to their ED. Two different types of penile implants exist: inflatable (2- and 3-piece) or malleable

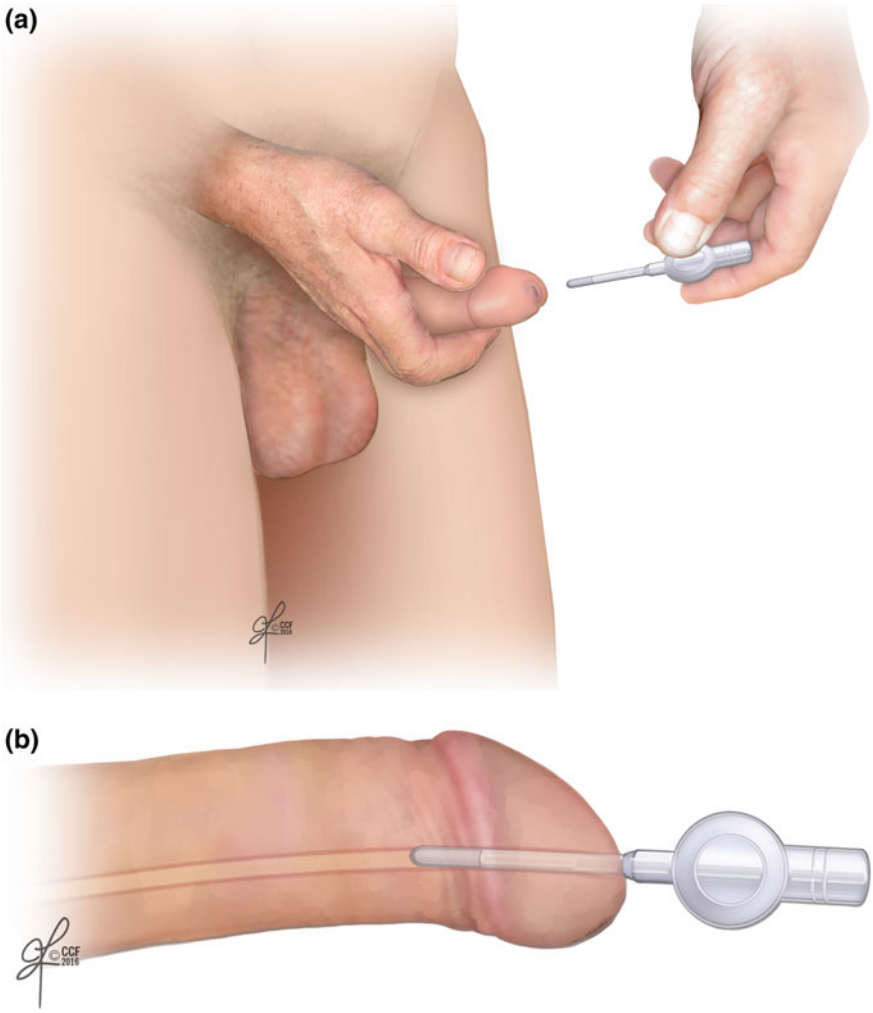

Fig. 13.5 Intraurethral PGE1. **a** Insertion of a medicated pellet. **b** Pellet inserted. © CCF 2016. Reprinted with permission

devices (Fig. 13.6) [83–85]. Inflatable devices are generally more preferred by patients as they provide more natural erections than malleable devices. When fully inflated, they provide superior rigidity because they completely fill the corpora cavernosa and when deflated they provide a much better flaccidity and concealment to the penis. Inflatable devices consist of two cylinders inserted inside the corpora cavernosa, a pump placed in the scrotum, and a reservoir that is either confined within the cylinders (in case of 2-piece devices) or as a separate component placed beneath the fascia of the lower abdomen (in case of 3-piece devices). Malleable devices, on the other hand, are semirigid rods that are implanted inside the

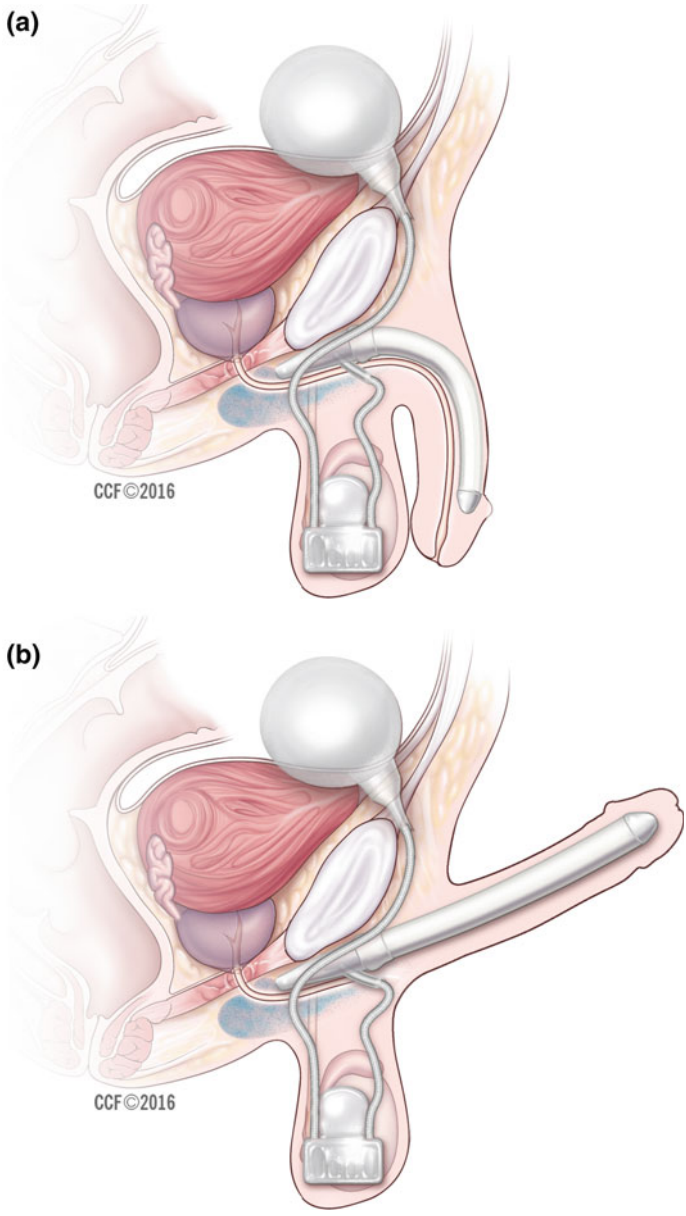

Fig. 13.6 Penile prosthesis implantation. **a** Deflated. **b** Inflated. © CCF 2016. Reprinted with permission

corporeal bodies. Despite providing a satisfactory rigid erection enough for penetration, their major drawback is the lack of concealment that happens as the penis stays in a semierect condition.

Surgical implantation is usually performed through a penoscrotal or an infrapubic approach. The penoscrotal approach is generally more favored as it allows better exposure of the corporeal bodies and also allows easy placement of the pump inside the scrotum. The only technical difficulty often encountered is the placement of the reservoir, which mandates blind placement into the retropubic space—a step that can be further complicated in patients with history of pelvic surgery. The infrapubic approach has the advantage of placing the reservoir under direct vision; however, implantation of the pump may be challenging and patients are at a slightly increased risk of dorsal nerve injury.

PPI has the highest satisfaction rates as a treatment modality for ED. Nearly 100% of patients and 95% of partners reported a satisfactory outcome with PPI [86–89]. Long-term follow-up studies have confirmed a consistently favorable outcome. One multicenter study followed 372 men for 48 months and revealed that 79% of patients were using their device at least twice monthly and 88% would recommend the prosthesis to a friend or relative [90]. In another study of similar design, 185 patients were followed for 59 months. Almost 93% of patients were using the prosthesis an average of 1.7 times weekly with excellent satisfactory results [87].

Complications of penile implant surgery include infection, malfunction, erosion, migration, sizing problems, and auto-inflation. Infection occurs in approximately 2–3% of cases and is considered a devastating consequence. Treatment entails removing all components of the prosthesis and re-implanting a newer device, months later. Re-implantation in this case is, however, considered to be technically difficult due to excessive fibrosis that would develop during the healing period. In an effort to overcome this outcome, Mulcahy et al. have established a salvage procedure where in up to 80% of salvage patients the secondary implant was preserved [91]. Antibiotic impregnated prosthetic devices were developed to further reduce the incidence of postoperative infection [65]. Populations at higher risk of infection include patients undergoing revision surgery, immune compromised, diabetic, and spinal cord injured patients [83, 85].

As with any other mechanical device, penile prosthesis can wear off with repetitive use. Despite that, the reported mechanical survival of inflatable penile prosthesis after 15 years of use is about 65% [92]. Further, technical modifications resulted in a mechanical failure rate of less than 5% after 5 years follow-up [90, 93]. Patients may experience modest penile shortening after PPI, which should be conveyed during counseling prior to surgery.

Conclusion

The management of erectile dysfunction requires a thorough understanding of its different pathophysiologies and presentations. Clinicians should hold a degree of intuition to identify at-risk patients who would benefit from cardiovascular risk

reduction. Several diagnostic modalities are available, each with a specific indication to help in treatment planning. Generally, a stepwise approach to therapy is favored to minimize unwanted side effects and achieve a satisfactory sexual life.

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Chapter 14
Spinal Cord Injury Related Infertility

Michael S. Floyd Jr., Sanjeev Sharma and Gurpreet Singh

Introduction

Spinal cord injury (SCI) is a traumatic, life-changing event with significant permanent physical, psychological, and emotional sequelae for the patient regarding mobility, independence, loss of motor and sensory function and bladder and bowel control. With improvements in bladder management, including intermittent catheterization and appropriate use of antibiotics, morbidity and mortality related to bladder and renal dysfunction have significantly reduced. Morbidity related to the bladder and kidney, however, still remains the commonest reason for patients being readmitted to spinal units [1].

Spinal cord injury demographics are changing. In the Northern Hemisphere, recent studies have reported an increased prevalence of injuries in older patients due to falls [2]. Over a 20-year period, the mean age at injury has increased, yet men still account for 75% of injuries. The decline of industries such as mining and an increased life expectancy may explain this pattern. Canadian studies have attempted

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to address the exact incidence of SCI and found incidences of 1785 per year in 2010 [3]. Australian-based research has found that the greatest prevalence is in men between the ages of 40–64 and cites an incidence of between 21 and 32 cases per million per year [4]. In Scandinavia, the demographic shift in spinal cord injury over a 25-year period has been attributed to the increasing influence of osteoporosis on senior citizens health [5]. Elderly patients are more likely to have cervical spine injuries due to falls, and, although they are less likely to undergo surgical intervention, they exhibit higher rates of in hospital mortality [6].

Aside from road traffic accidents, falls, and industrial accidents, specific leisure activities have been extensively studied in relation to SCI. Studies on mountain biking [7], skiing [8], rugby [9], ice hockey [10], diving [11], and paragliding have all been reported [12]. Suicide-related injuries have also been studied [13]. Isolated studies on esoteric causes of SCI also exist [14].

The psychological impact of such a life-changing event should not be underestimated; patients use a variety of coping strategies, yet sexual well-being tends to be neglected. Studies have now shown that SCI has a definite negative impact on quality of life (QOL) across a range of dimensions, such as physical and social functioning, mental health, vitality, emotional expression, and impaired sexual capacity [15]. Although the frequency of sexual activity may decline after injury [16], the desire for intimacy remains [17]. It is, however, recognized in the SCI patient that the primary reason to engage in sexual activity alters with the prime aim being a desire for intimacy, followed by the willingness to keep a partner with fertility being less important [18]. Studies examining the effects on sexual function in the SCI men have shown that (1) the commonest reason to ejaculate is for pleasure and sexual intimacy, (2) neuroplasticity has a role in sexual function post injury, and (3) up to 60% of respondents have tried methods of improving erectile function [19].

Infertility affects approximately 90–95% of SCI men [20, 21], with only 5% able to conceive unaided [22]. The two main reasons are (1) ejaculatory dysfunction, which is permanent in between 85 and 97% of men, and (2) poor quality semen [20, 21, 23]. In SCI men, the ability to retain erections is preserved better when compared to ejaculatory function [15] as 80% of SCI men will have some erectile function up to two years after their injury. However, erectile function must not be used as a benchmark of sexual or reproductive potential in SCI men.

There is limited data pertaining to sexual function in female SCI patients. Specific questionnaires such as the 104-item Spinal Cord Injury in women questionnaire have been devised in an attempt to understand specific aspects of female sexuality following spinal injury [24]. The authors reported that although up to 80% of respondents engaged in sexual intercourse following injury [24], reasons for abstaining included poor libido and lack of self-esteem. In the immediate aftermath of a spinal cord injury, female patients lose the ability to have reflexive sexual responses [15], and amenorrhea may persist for up to 12 months following injury [25].

In the female SCI patient, physical difficulties with arousal combined with a fear of autonomic dysreflexia and spasticity pose barriers to sexual function. Specific

problems include loss of vaginal lubrication [15] (which requires an intact S2–S5 segment) and fear of coital-related incontinence [18]. However, fertility-related problems are much less common than in SCI men, as conception rates approach that of the ambulant population [25]; there are specific pregnancy-related issues such as premature labor that can result in prolonged hospital stays [26]. Higher rates of autonomic dysreflexia, pressure ulceration, catheter expulsion, and Caesarean delivery are also experienced by pregnant SCI patients [27].

Pathophysiology

Following spinal injury, men experience difficulties with erections, ejaculation, and additionally sperm quality is affected, culminating in sub-fertility. The level and degree of completeness of spinal cord injury are important determinants of sexual functioning [15]. It is important to review the neurophysiology of erectile function and ejaculation prior to discussing the effects of spinal cord injury on male sexual and reproductive function.

The nerve supply to the penis has somatic and autonomic components. The sensory and motor nerve fibers of the somatic component are carried in the dorsal nerve of the penis, which is a branch of pudendal nerve (S2–S4). The somatic nerve fibers innervate the external sphincter, bulbospongiosus, and ischiocavernosus muscles as well [28, 29].

The sympathetic fibers originate from segments T10–L2; the preganglionic fibers ascend through the inferior mesenteric and superior hypogastric plexus. From there, the fibers travel via the hypogastric nerves. In the sacral area, the postganglionic sympathetic fibers to the penis run through the pelvic and pudendal nerves. The parasympathetic preganglionic fibers originate in the spinal segments S2–S4 and reach the pelvic plexus through the pelvic nerve. The postganglionic fibers reach the penis through the cavernous nerve [28, 29].

Erection

There are two kinds of erections: reflex and psychogenic. These are predominantly under parasympathetic control with contribution from the sympathetic centre in psychogenic erections [28–30]. Reflex erections are initiated by direct or tactile stimulus of the genital region through the intact sacral reflex arc, located in the parasympathetic center (S2–S4). Psychogenic erections are initiated by erotic stimuli independent of direct stimuli and are mediated via the thoracolumbar center in the spinal segments T10–L2. The sympathetic centre in T10–L2 is also responsible for the maintenance of erection once the process has been initiated. The key mediator of erection is vasodilatation and opening of the arteriovenous shunts

in the corpora cavernosa. Smooth muscle relaxation of the penile arteries in the corpora cavernosa leads to erection and is assisted by neurotransmitters such as nitric oxide [31].

In summary, the parasympathetic center (S2–S4) is predominantly responsible for mediating reflex erections but the psychogenic erection is mediated through the center in spinal segments T10–L2 [25, 28–30].

In complete upper motor neurone (UMN) lesions above spinal segment T10, the patient can still have reflex erections but not psychogenic erections. In a lower motor neurone (LMN), injury to the sacral roots leads to failure of reflex erections but psychogenic erections can still occur. However, in patients with an incomplete injury to the T10–L2 segments, there is considerable degree of variability, and hence the patients have capacity for both psychogenic and reflex erections [28, 29].

There is a variable period of spinal shock immediately after a spinal cord injury leading to complete lack of erections, both psychogenic and reflex. Following recovery from spinal shock, the patient can demonstrate one of four scenarios. In the first scenario, an UMN injury above T10, the patient can have reflex erections with engorgement of both corpora cavernosa and corpus spongiosum [30].

The second scenario is that of psychogenic erection. However, if the injury is UMN and below T10 but above the L2 spinal segment, the erection is only partial as there is no engorgement of corpus spongiosum.

The third scenario is when the injury is between L2 and S2 and there is no damage to S2–S4: The patient may have both psychogenic and reflex erections.

The fourth scenario is an LMN injury to sacral roots S2–S4; this leads to failure of reflex erections but not psychogenic erections [30].

There is, however, a considerable variability around these broad pathophysiological outcomes as neuroplasticity plays a large role.

Ejaculation

Ejaculation like erection is a coordinated process involving the autonomic and somatic nervous systems. The process of ejaculation consists of two parts: emission and expulsion. The first part starts with the afferent input received through the dorsal nerve of penis, which leads to the activation of efferent sympathetic fibers arising from spinal segments T10–L2 [28–30]. These nerve fibers travel via the hypogastric nerve plexus to the seminal vesicles and prostate. There is a coordinated contraction of the epididymis, vas deferens and ejaculatory ducts. The emission phase ends with the deposition of sperm and ejaculatory fluids in the posterior urethra. There is also concomitant closure of the bladder neck [31].

The second phase of ejaculation is expulsion, which is mediated via parasympathetic center in segments S2–S4. Expulsion is effected via two neural pathways. The first is via the pelvic nerve, which leads to stimulation of prostate and seminal vesicles, whereas the second is via the somatic system through the perineal branch of the pudendal nerve. The stimulation of the somatic system leads to the

Table 14.1 Ejaculation rates according to the type of injury [30]

Type of injury	Ejaculation rate (%)
Complete upper motor neuron	4–11
Incomplete upper motor neuron	32
Complete lower motor neuron	18
Incomplete lower motor neuron	70

contraction of the bulbocavernosal and ischiocavernosal muscles as well as the relaxation of the external urethral sphincter [32].

The combination of these two neural pathways leads to a forceful pulsatile antegrade ejaculation. Closure of the bladder neck prevents retrograde ejaculation [27]. In summary, ejaculation requires integration of spinal segments T10–L2 and S2–S4; the former for emission and closure of bladder neck and the latter for expulsion.

Injury above level T10 can spare the sacral arc; hence, the patient can ejaculate. However, injury below T10 has variable effects depending on the level and completeness of the injury. UMN and LMN affect the ejaculation differently (Table 14.1) [30].

Disruption of the reflex arc causes anejaculation: an absolute lack of ejaculate despite normal sexual stimulation due to failure of the emission phase of ejaculation [33]. Spinal cord injury is the commonest cause of neurogenic anejaculation [33].

Sperm Retrieval in Men with SCI

After the initial period of spinal shock, 85% of men will have some degree of erectile function, and 2 years after their injury only 10% of SCI men are able to ejaculate successfully during intercourse [34].

Historically, anticholinesterases were the first class of drugs used to assist with ejaculation. Guttman, in the early seventies, originally used intrathecal prostigmin to induce ejaculation in SCI men with some success, but the use of the drug was discontinued due to significant side effects including autonomic dysreflexia [35]. Subcutaneous physostigmine with concomitant administration of *N*–butylhyoscine was described by Chapelle et al. [36], but again discontinued due to parasympathetic side effects. Despite its undesirable side effect profile, there are reports of successful pregnancies in paraplegic men following intrathecal neostigmine administration [37]. Prostatic massage is also another simple, inexpensive, noninvasive method for obtaining ejaculatory fluid from neurogenic anejaculatory patients, and successful pregnancies have been reported [38, 39].

The non-pharmacologic methods used for anejaculation consist of (1) assisted ejaculatory techniques—penile vibratory stimulation (PVS) and electro-ejaculation (EEJ); and (2) methods where the sperm are directly collected from the testis—surgical sperm retrieval techniques, e.g., percutaneous epididymal sperm aspiration (PESA), testicular sperm aspiration (TESA), testicular sperm extraction (TESE), and others.

Penile Vibratory Stimulation

PVS was first described by Sobrero in 1965 [40] and is one of the simplest methods used for collection of sperm in SCI men.

Technique

A special vibrator (Ferticare Personal) is placed on the penile base or glans and vibration applied until ejaculation occurs. Typically, the procedure is stopped every 5 min to assess for any penile skin changes. It works best in men with lesions above T10 or incomplete lesions and requires intact dorsal penile nerves and a reflex arc. It is contraindicated in cardiac disease or untreated hypertension as autonomic dysreflexia may occur. Severe irritation of the glans penis is a relative contraindication, and extreme care should be taken if the patient has a penile prosthesis [41]. (In our center, we do not recommend this for patients with a penile prosthesis.) Proper positioning of the patient (usually at 45°) and bladder preparation are required with prompt drainage of the bladder and instillation of a buffering agent prior to initiation of PVS. Suprapubic catheters should be clamped, the urethral catheter should be removed, and an appropriate sterile device held at the external meatus to facilitate collection of ejaculate. As a technique, it has practical advantages over electro-ejaculation as it can be administered at home with partner involvement and cooperation, enhancing the sexual experience, is relatively inexpensive, and does not require an anesthetic [30]. With time, it can be patient administered and instead of special vibrators, many patients report success with generic vibratory sex devices.

Prognostic Factors

The most important factor that can predict success with PVS is the presence of an intact T10–S4 spinal segment and an intact dorsal nerve of penis. Presence of hip flexion reflex (L2–S1) and presence of bulbocavernosus reflex are also good predictors of success. Lesions below T10 and men with absence of hip flexion and bulbocavernosus response are associated with poor response. PVS is less successful in men within 6–18 months post injury [30].

Success

In optimal patients (injuries above T10), PVS is successful in up to 85% patients. In patients with an injury below T10, the success rate falls to 15%. In patients who have a favorable response, two-thirds are likely to have antegrade ejaculation and one-third have retrograde ejaculation [30]. PVS is a suitable technique for sperm

Table 14.2 Ejaculatory rates with penile vibro-stimulation

	Specimen					
Method	No. of procedures	Antegrade alone	Retrograde alone	Combined	No specimen	Success rate
Penile vibratory stimulation	14	9	0	2	3	78.5%

retrieval for any SCI patient, and the best results are obtained with higher amplitude vibrators [40] and in injuries above spinal level T10. Use of a higher amplitude vibrator has been shown to yield semen samples in up to 95% of patients with an intact lumbosacral cord [42]. Sperm quality however, is variable (Table 14.2).

Electro-Ejaculation

EEJ is an alternative method for sperm collection and can be used for similar indications as PVS. EEJ is, however, much more invasive, as it requires medical intervention and the patient may require general or spinal anesthesia [30].

Electro-ejaculation as a technique was originally designed to assist in animal husbandry and was first described in the 1930s [43]. In humans, the technique was first described by Horne in 1948 [23] and became an established technique for assisted reproduction in the SCI patients in the late 1980s [44]. The first reported pregnancy as a result of electro-ejaculation was reported by an Australian group in 1975 [45], and later in Japan in 1992 [46].

Technique

A trained physician in a theatre environment, usually using general anesthesia, must perform electro-ejaculation. A spinal anesthetic may also be used with lesions above T10. The patient is placed in the lateral decubitus position, and the bladder is temporarily catheterized to allow emptying and permit insertion of buffering solution into the bladder [30].

A rectal probe is inserted and voltage progressively increased in 1–5 v increments, which eventually stimulates the peri-prostatic and peri-rectal sympathetic nerves until ejaculation occurs [32]. The probe temperature is monitored throughout the procedure and the procedure suspended if the temperature rises above 40 °C. During emission, the sympathetic nerves cause closure of the internal sphincter, preventing retrograde ejaculation and concomitant peristalsis of the ductus deferens. Stimulation with EEJ also leads to parasympathetic peristalsis of the urethral musculature, and contraction of the bulbospongiosus allowing emission of semen [47].

Seminal emission depends on the level and completeness of the lesion, and a significant amount of sperm may be expressed into the bladder. Urethral milking may be required (with an assistant) as penile erection may not always occur with electro-ejaculation [48]. The antegrade fraction is collected in a sterile container held by an assistant at the meatus. Postoperatively, the bladder is again catheterized to obtain the retrograde fraction, which can be centrifuged. Rectal pathology is a contraindication, and patients with lesions above T6 are prone to dysreflexic episodes and may require calcium channel blockage at induction, or a spinal anaesthetic.

Prognostic Factors

The indications for EEJ are similar to PVS. However, EEJ is successful in injuries below T10, and the technique can be used successfully in the absence of hip flexion and bulbocavernosus reflexes. EEJ can also be used within six months of the injury, and the level and completeness of the injury are not predictors of success. The overall success is similar at all levels [30], but when compared to PVS, electro-ejaculation is a more expensive procedure and must be done in a clinical environment.

Success

Results from one of the earliest large studies demonstrated that 93% of patients with upper motor neuron lesions achieved antegrade ejaculation via this method with 63% of lower motor neuron patients also achieving it [46]. Aside from SCI, it can be successfully used in other forms of iatrogenic anejaculatory dysfunction, such as following retroperitoneal surgery for disseminated testicular cancer [49]. The quality of sperm is often suboptimal and in our experience improves with control of infection and frequent ejaculations. The data for our centre has been summarized in Table 14.3.

Table 14.3 Ejaculatory rates with electro-ejaculation

	Specimen					
Method	No. of procedures	Antegrade alone	Retrograde alone	Combined	No specimen	Success rate
Electro-ejaculation	96	3	30	55	8	91.6%

Surgical Sperm Retrieval

Prior to the introduction of sperm retrieval techniques, donor semen was the only method of achieving pregnancy available to azoospermic men. The introduction of surgical sperm retrieval techniques revolutionized the management of sub-fertility in men with SCI. Men in whom no ejaculate can be obtained or whose ejaculate contains no viable sperm require more invasive forms of intervention: surgical sperm retrieval. Sperm can be obtained through various techniques from either the epididymis or testis by percutaneous aspiration, biopsy, or formal surgical exploration [33]. In SCI men, both testicular sperm aspiration (TESA) and testicular sperm extraction (TESE) are used for sperm extraction.

Technique

Testicular sperm aspiration (TESA) can be done under local anesthesia. A needle is inserted into the testicular parenchyma directly through the scrotal skin. The upper, interpolar and lower poles of the testis are sampled with a 19-gauge needle. Single or multiple punctures can be used. Side effects include hematoma formation.

TESE is another method of sperm retrieval. Testicular sperm extraction involves incising the tunica albuginea, applying pressure to the testis and retrieving the extruded tissue. Single site TESE, multiple site TESE, and micro-TESE have all been described [50]. Like TESA, it is a blind technique and cannot identify the active sperm-producing areas of the excised tissue until tissue has been extracted from the patient [51]. Micro-TESE is a microsurgical technique that aims to identify seminiferous tubules more likely to contain viable spermatozoa. Under a microscope, tubules containing developing germ cells rather than Sertoli cells in isolation will appear larger and more opaque than tubules without [51].

In our center, we now exclusively use TESE as a method of surgical sperm retrieval and dependent on testicular volume, we may explore one or both testes. This has superseded TESA, as sperm quality and quantity in spite of multiple punctures was very variable with TESA.

Prognostic Factors

Surgical sperm extraction techniques are suitable for all SCI patients. The techniques can be used irrespective of their level or completeness of the injury. These techniques can also be used in the first six months post injury, as well as (a) in patients with low volume testes, (b) in all forms of bladder management and (c) in patients with frequent recurrent infections.

The main disadvantage of surgical sperm retrieval techniques is that the amount of sperm obtained is very small; hence, the female partner eventually requires

Table 14.4 Retrieval and fertilization rates according to etiology

Etiology	Retrieval rate	Fertilization rate (%)
Obstructive azoospermia	141/141 = 100%	79.1
Non-obstructive azoospermia	27/60 = 45%	54.2
Spinal cord injury	11/11 = 100%	100

in vitro fertilization (IVF) followed by intracytoplasmic sperm injection (ICSI). As sperm retrieval is done independent of the female partner the sperm specimen is cryopreserved.

Success

The success of these techniques is comparable to the success rates in the able-bodied patients. The data from our centre is summarized in Table 14.4.

Sperm Transport and Storage

Our centre is based in Southport and Ormskirk National Health Service (NHS) Trust, Southport, Merseyside, United Kingdom. It is a district general hospital that provides secondary level care for the local population. The North West Regional Spinal Injury Unit is also based in the hospital. Assisted conception units with facilities for IVF, ICSI, and other advanced fertility techniques are located in regional tertiary centres. Our regional centre is based in Liverpool Women's Hospital, 30 miles away.

This model, with a spinal injury unit and separate assisted conception unit being located in two different hospitals, is not unusual in the UK. However, it creates unique problems for the management of infertility in spinal cord injury patients. The challenge arises because the sperm retrieval is performed in the spinal unit and then the sperm specimen is transported to the assisted conception unit for analysis, cryopreservation and eventual treatment.

The management protocols for our patients have been established over the last 25 years and consist of an integrated pathway that includes referral, assessment, sperm collection and assisted conception for spinal patients undergoing fertility treatment. In the UK, assisted conception treatment of infertility is done under the guidance of the Human Fertilization and Embryology Authority (HFEA). The treatment protocols in our center fulfill the HFEA criteria [52].

Following surgical sperm retrieval, the specimen is immediately transported by car in G-Mops™ Plus (Vitrolife, Göteborg, Sweden) [52] culture medium to the assisted conception unit where the specimen is then assessed for sperm parameters and then cryopreserved. Post-thaw analysis is performed a few days later to confirm viability.

In our center, we only use cryopreserved sperm for the subsequent treatment, which is IVF/ICSI. We have not found any deterioration in the fertilization rates in the SCI patients compared to the sperm from the able-bodied men (Table 14.4).

The advantages of the model described include the following: (1) collection of sperm is done according to the suitability and convenience of the spinal injury patient, and (2) the outcome is not affected by either the level or type of injury. In addition, the assisted conception can be scheduled to suit the couple. Sperm collection and cryopreservation can also be done for single men for future use as well.

Semen Analysis

There are well-defined and significant differences in the seminal quality and the seminogram of the men with SCI, when compared to able-bodied, ambulant men, and this is proposed to be multifactorial. Typically, volume may be normal but frequently discolored—a condition known as "rusty pipe syndrome of unknown etiology." [20]. Specimens of ejaculate from spinal patients typically have an increased count but have poor quality, specifically for motility and viability [25, 53]. Changes in seminal parameters occur as soon as two weeks after acute injury [54]. Early work in the field of SCI related fertility identified various factors thought to contribute to poor semen quality in SCI men, namely urinary tract infections, method of bladder management, static prostatic fluid, testicular hyperthermia and histological changes, hormonal alterations, presence of antibodies, and use of chronic medications [55]. Hirsch et al. [56] proposed that testicular failure and accessory sexual gland dysfunction may also be responsible for poor semen quality in spinal cord injury patients. Method of bladder drainage has been shown to influence the amount of motile spermatozoa with intermittent self-catheterization being the optimum method that is "sperm friendly." [57]. Additionally, level of neurological insult is also important and demonstrates a linear relationship with caudal injuries demonstrating the most deleterious effects on motility [22].

The World Health Organization (WHO) criteria for assessment of semen are considered the gold standard for the assessment of semen analysis (Table 14.5) [58].

Table 14.5 World Health Organization (WHO) reference limits for semen analysis (WHO—5th edition [58])

Criteria	Reference limits for semen analysis
Volume (ml)	1.5 ml
Total sperm (millions in ejaculate)	39 million
Sperm concentration (millions per ml)	15 millions per ml
Total motility	40
% normal morphology	4%

Table 14.6 Quantitative semen parameters in the ejaculates of the spinal injury patients

	Volume (mls) (May include culture media)		Concentration/ml		Motility (%)		Cells (HPF)	
	Range	Average	Range	Average	Range	Average	Range	Average
Antegrade	0.4–20	7	0.2–350	111	1–89	15	1–11	4
Retrograde	1–80	25	0.4–450	44	1–15	4	13–26	15

However, the same criteria cannot always be applied to the semen specimen obtained from a spinal injury patient because of the unique circumstances that apply to this patient cohort. These include the following: infrequent ejaculations, the need to keep the specimen from entering the bladder and the requirement to instill culture medium intravesically. The results from our center are described in Table 14.6, and when compared to the WHO reference limits (Table 14.5), it is obvious that all the parameters do not conform.

Case Discussion and Presentation

Spinal cord injury typically occurs in men at the peak of their reproductive potential (with the average age at injury being 33.4 years) and remains the commonest cause of neurogenic ejaculatory dysfunction [29, 33]. Additionally, altered seminal quality and erectile dysfunction contribute to fertility concerns in the SCI man. The natural ability to conceive is altered due to sacral autonomic disruption [47], and, therefore, neurogenic ejaculatory failure is of paramount importance when dealing with fertility concerns in a spinal cord injury patient. Infertility affects more than 90% of men with SCI [20]. As most men are anejaculatory, they commonly present to a urologist for fertility advice, treatment of erectile dysfunction, or sperm retrieval [28].

Although reflex erections still occur in spinal cord patients, they are usually not of a sufficient duration to permit intercourse. However, because the vascular mechanisms are intact, treatment of erectile dysfunction in the spinal cord-injured men is similar to the treatment strategies used in the ambulant male with phosphodiesterase type 5 (PDE5) inhibitors, intracavernosal injections, vacuum devices, and penile implants (see Chap. 13) [59]. Due to altered sensation in the spinal patient, the use of inflatable implants over rigid implants is favored as it reduces the risk of implant erosion. Therefore, erectile dysfunction alone does not pose difficulties for sperm retrieval [59].

As previously discussed, sperm retrieval is facilitated by assisted ejaculation via penile vibratory stimulation (PVS) or electro-ejaculation (EEJ). In the acute phase,

immediately post injury normal semen can be retrieved from a SCI injured man via EEJ, whereas semen retrieved by assisted ejaculation in the chronically injured SCI man is of abnormal quality, with reduced motility [33].

Surgical sperm retrieval is performed by either open or percutaneous biopsy (TESA,TESE,PESA), or microdissection TESE. There are no randomized or cohort studies comparing the success rates of these methods in SCI men [59].

Semen quality in SCI men is typically normal in concentration, but low in motility and viability and contains higher numbers of inflammatory cytokines. Despite adequate retrieval techniques, poor semen quality remains an issue. Although testicular hyperthermia, abnormal testicular histology, type of bladder management, urinary tract infection, changes in the hypothalamic pituitary axis, and anti-sperm antibodies have been postulated as etiological agents causing poor semen quality, it is accepted that the cause is multifactorial [55].

Obvious lifestyle changes occur following a spinal cord injury. It had been thought that prolonged sitting in a wheelchair with legs close together induced scrotal hyperthermia, which was deleterious to semen quality [60]. A study examining scrotal and thigh temperature in SCI and ambulant men found no difference [60]. Brackett et al. [61] have reported that scrotal temperature does not contribute to altered semen quality in SCI men nor does it cause elevated gonadotrophin levels. Length of time since initial neurological insult has no major effect as sperm quality remains the same during the chronic phase of the injury [62].

Bladder management changes radically following spinal injury and it has also been postulated that methods of bladder drainage can have negative impacts on semen quality. Several studies have demonstrated that use of intermittent catheterization leads to improved sperm motility, compared with other bladder management modalities [22] but no method (even spontaneous voiding) is associated with normal semen quality. It is thought that high pressure voiding in a neuropathic bladder allows urinary reflux into the ejaculatory ducts, vas deferens, and seminal vesicles, which negatively impacts sperm quality [57]. Any modality of optimizing bladder drainage to ensure low-pressure emptying and a reduction in infections may enhance fertility in the spinal patient [22]. Even in ambulant SCI men (American Spinal Injury Association [ASIA] classification A), the urological sequelae of a spinal injury are significant, with up to 68% of patients having urodynamic evidence of altered bladder function despite otherwise good functional neurological recovery [63].

It is acknowledged that sperm can be obtained easily by non-surgical means from most SCI men [64]. It is increasingly recognized that there is a growing trend toward surgical sperm retrieval being relied on as a primary measure in SCI men, even though sufficient numbers of motile sperm are present in an ejaculated specimen [29]. Recent studies have proposed a stepwise algorithm for sperm retrieval in men [59] and suggest a trial of vibratory stimulation, followed by prostatic massage, and then EEJ if unsuccessful.

Case-Based Discussions

Only 5% of SCI men are able to conceive naturally without assistance [65]. In 1999, Marina et al. reported a case of a triplet pregnancy in a 27-year-old paraplegic man in whom assisted ejaculation techniques failed. The couple successfully conceived with sperm obtained through prostatic massage [39]. These are unusual success stories; we describe three clinical scenarios in patients with different injury levels with variable success.

Case 1

A 21-year-old British male sustained C5–6 complete tetraplegia in 1998, following a diving accident. Since 2006, this patient had been managing his bladder by reflex voiding into a convene catheter and had no problems with infections or incontinence. He presented to our joint infertility clinic in March 2010 requesting treatment. The patient's partner was 28 with one previous child, and her investigations were normal.

The couple had been trying for a child for about 18 months through penile vibratory stimulation and vaginal sperm insemination. The patient was a non-smoker and there were no significant comorbidities. The patient's testicular volume was 19 and 20 mls, and gonadotrophin measurements were normal.

After discussion with the couple and obtaining informed consent, the patient was scheduled for electro-ejaculation under general anesthetic. Antegrade sperm was collected from the meatus, and retrograde sperm was collected via urethral catheter retrieval. Sperm was transported to the fertility center, but the sperm obtained was of poor motility and deemed to be unsuitable for uterine insemination or IVF. EEJ was repeated on a further two occasions at 3–4 weekly intervals, and although the sperm quality improved, IVF was unsuccessful. A decision was taken to proceed with ICSI as it was felt that there was enough reasonable quality sperm to ensure a chance of success. On the fourth attempt with EEJ, ICSI resulted in successful fertilization and pregnancy.

Case 2

A British, Caucasian male, born in 1968, sustained complete paraplegia at T4 level in March 1988 due to a fall from a ladder while working on an overhead drain. He had an indwelling urethral catheter during the acute stage of his injury and subsequently managed his bladder via convene sheath drainage. In 1999, he underwent implantation of Medtronic SynchroMed pump for intrathecal delivery of baclofen. He suffered with recurrent urine infections and underwent assessment with bladder ultrasonography and video urodynamics. Following this, he was started on anticholinergic pharmacotherapy and a clean intermittent self-catheterization regime

with symptomatic improvement. Compliance with bladder management remained suboptimal as occasional infections remained a problem.

Reflex erections were possible, augmented with PDE5 inhibitors. The patient's partner was 38 years old with two previous children and had been fully investigated with nothing untoward detected. He was anejaculatory. His gonadotrophins were within normal limits, and his testesterone levels were at the lower limit of normal at 9.5 ng/ml. The patient's testicular volume was 14 and 15 mls. After a discussion and informed consent, the patient was approved for EEJ. This was done under spinal anesthetic, due to the risk of autonomic dysreflexia. Both the antegrade and retrograde specimen remained azoospermic in spite of two attempts. Further management was discussed with the couple and the patient was scheduled for a TESE, done under a general anesthetic. TESE was successful with good quality sperms retrieved, and IVF/ICSI was successful.

Case 3

A 28-year-old Caucasian male sustained multiple injuries including rib and pelvic fractures and paraplegia at L1/2 level following a road traffic accident in 2012. His bladder management was erratic with a combination of clean intermittent self-catheterization (CISC) and indwelling urethral catheters, a hypocompliant bladder, and recurrent urinary tract infections, but his upper tracts were stable.

The patient presented with his partner, a healthcare worker, requesting fertility treatment. Intracavernosal injections were used to achieve satisfactory erections. They had no children, and all female investigations were normal. The patient's serum testosterone was normal. The patient was anejaculatory and following discussion and informed consent, the couple elected to proceed to TESE under general anesthesia. This was successful with good quality motile sperm retrieved from both testes, which were cryopreserved for future ICSI.

Discussion

All patients with SCI suffer from various degrees of infertility due to erectile and ejaculatory dysfunction. Management of these is tailored according to patient needs, expectations and level of injury. In our center, patients are encouraged with sexual rehabilitation soon after the injury with erection aids and ejaculatory help. Fertility is discussed, and we have had a variable success with PVS and EEJ. Sperm parameters tend to be poor despite repeated ejaculations.

The aim of treatment should be to help patients and should accommodate their other rehabilitation requirements. Ideally, a fresh sperm specimen used for ICSI results in the best possible chance of success (up to 64%) [66]. Most SCI men, however, are not thinking of starting a family immediately following injury and frequently explore the option of freezing the sperm for future use. This reduces the

chances of success. Sperm retrieved surgically gives the SCI patient the best chance of success following cryopreservation with sperm parameters comparable to the able-bodied patient and similar success rates of fertilization and embryo quality.

In our center, once home insemination techniques and uterine insemination fail, we offer the patient the options of PVS, EEJ and surgical sperm retrieval where the advantages and disadvantages are discussed in a designated clinic.

Unsurprisingly, an increasing number of patients are opting for surgical retrieval with excellent success.

Management

Following a SCI, the frequency of intercourse may decline [67] yet the need for sexual expression remains [15]. Patterns of sexual behavior adjust following injury and when measured by the Sexual Interest and Satisfaction scales are positively correlated with a better quality of life [68]. Anderson et al. [69] studied the recovery priorities of spinal injury patients and noted that regaining sexual function and fertility was the most important function out of seven assessed in the paraplegic patient.

SCI patients need assistance with semen collection for management of their fertility, and PVS and EEJ are the two oldest methods that have been in use since the 1930s [43].

Men in whom no ejaculate can be obtained or whose ejaculate contains no viable sperm require more invasive forms of intervention: surgical sperm retrieval. Sperm can be obtained through invasive techniques from either the epididymis or testis by percutaneous aspiration, biopsy, or formal exploration. A drawback with these methods is that the yield of sperm is relatively low and requires assisted conception [33] following surgical sperm retrieval.

Testicular sperm extraction is our preferred method of surgical sperm retrieval. Single site TESE, multiple sites TESE, and micro-TESE have all been described [50]. Micro-TESE is a recent microsurgical technique that aims to identify seminiferous tubules likely to contain viable spermatozoa [51].

Sperm collected by either direct retrieval or assisted ejaculation can be used for assisted reproduction. The choice of technique depends on patient characteristics and preferences. Specific to male factor infertility, the main determinant is the degree of motile sperm in the semen sample obtained [33]. Additionally, female factors, cost and the ability of the couple to cope with conception failures are relevant considerations [41]. The time delay between sperm collection and subsequent insemination is also relevant. Frequently, the two procedures are done in separate institutions by both urological and gynecological services. In intravaginal insemination, the ejaculate is injected intravaginally with a syringe at the time of ovulation. Intrauterine insemination involves isolating the fraction of motile sperm first and then injecting them directly into the uterine cavity. This can be supported with hormonal therapy in order to maximize the chance of successful implantation.

In in vitro fertilization, oocytes and sperm are mixed in a dish to permit fertilization. ICSI involves direct injection of sperm into the oocytes, which have been prestimulated. After 5 days of in vitro development, the embryos are implanted into the uterus [70].

Specific to the fertility in the spinal cord injured patient, multiple authors have attempted to define the incidence of the success rates following assisted reproduction techniques as it varies between 25 and 70% [71–76].

Success remains a very difficult outcome to define as further studies with smaller numbers have shown similar pregnancy rates between sperm obtained from electro-ejaculation, TESE, and prostatic massage [77]. The first pregnancy as a result of collected epididymal sperm and subsequent IVF was reported in 1985 [78], whereas successful pregnancy due to ICSI was only reported in 1993 [79]. The important message for clinicians involved in fertility management of this complex but highly informed and motivated group of patients is that any treatment should be planned following detailed discussion with the couple, and success has to be measured against very limited expectations.

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Chapter 15
Establishing and Managing a Sperm Bank

Grace M. Centola

Introduction

Cryopreservation and storage of male reproductive cells and tissue has been a routine procedure to preserve the male gametes since the earliest reports of sperm freezing by Spallanzani and Mantegazza in 1866 [1, 2], with initial reports of human semen cryobanking in the 1950s by Sherman and colleagues [1, 3]. The first case of a human pregnancy from frozen sperm was reported by Bunge and Sherman in 1953 [4]. Since these earliest reports, thousands of children have been born following insemination with frozen–thawed semen. Male reproductive cells and tissue cryobanking is now a routine procedure for male fertility preservation for subsequent use in artificial insemination and assisted reproduction.

Sperm banking is recommended both for medical and for personal reasons, including prior to radiation or chemotherapy treatment for cancer, surgical sterilization (vasectomy), cytotoxic treatment for non-malignant diseases, military deployment, hormonal treatment, gender reassignment surgery, and assisted reproduction [1, 5–7]. Unfortunately, not all patients who would benefit from sperm banking are actually referred for this service, either because the risk of infertility was not identified, or time pressures precluded referral to the sperm bank [7]. Additionally, cryopreservation of microsurgically retrieved testicular and epididymal sperm is now a routine procedure and has been demonstrated to yield pregnancy rates comparable to use of fresh sperm [8]. Semen cryopreservation is required by state and federal regulation for anonymous donor sperm, following extensive screening and testing of the donor and quarantine of the semen specimens for a minimum of 180 days [5, 9–11]. Fertility preservation has recently been recommended for men who are delaying procreation due to age since it has been

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suggested that there is an increased risk of autism, schizophrenia, and de novo mutations with increasing paternal age [12–15]. More recently, cryopreservation of spermatogonial stem cells from prepubertal adolescents may potentially be used in the future for procreation by autotransplantation of testicular tissue. The spermatogonial cells are retrieved prior to cytotoxic or surgical treatment, or in patients with Klinefelter's syndrome [16, 17].

This chapter will discuss the process of instituting and managing a sperm bank. Topics include donor sperm banking, client depositor sperm banking, laboratory techniques, and management. This chapter will conclude with clinical vignettes taken from the author's years of experience in this field.

Donor Sperm Banking

Cryopreservation and storage of donor sperm has been a standard practice since the late 1980s with the onset of acquired immunodeficiency syndrome (AIDS) and risk of transmission of communicable diseases in semen [18–20]. The requirement for extensive screening of sperm donors with a 180-day quarantine/storage period of banked sperm and donor retesting prior to release for clinical use has been required by state and federal regulation and professional guidelines [2, 5, 10, 11, 18–21].

Anonymous and Known Sperm Donors

There are 2 categories of sperm donors. Anonymous sperm donors are the majority of donors used clinically. In the USA, these donors are generally unknown to the recipient, although most recently, some commercial sperm banks have offered identity release of donors. Directed or known donors are "known" to the recipient and her partner if applicable. The sperm from a directed donor is directed only to a specific recipient and does not become part of the general sperm bank inventory.

In the UK, however, the Human Fertilisation and Embryology Authority (HFEA) has maintained since 1991 a record of all births from assisted conception from licensed UK fertility clinics [22]. Children born as a result of sperm donation have a legal right to donor information, including physical characteristics, birth year and place, ethnicity, information about the donor's own biological children, marital status, medical history, and identifying information [22]. Thus, the donor anonymity is eliminated. Any commercial sperm bank that ships donor semen into the UK must agree to the identity release, and the donors must sign a consent to the release of this information. In France, gamete donation has been anonymous, yet, in most other European countries, there has been a trend toward identity release [23].

Directed or known sperm donors must be screened and tested in the same manner as anonymous sperm donors [2, 5, 10, 11, 18–21], the only real difference being the fact that the donor is known to the recipient. Additionally, if a donor is

ineligible due to high-risk behavior, a known donor can still be used for insemination of the known recipient if approved by the sperm bank medical director [10, 11]. There would be no medical approval if there is positive serological testing, especially positive for human immunodeficiency virus (HIV), human T-cell lymphotropic virus (HTLV), or hepatitis [10]. However, the US Food and Drug Administration (FDA) allows use of specimens from a known sperm donor that tests positive for communicable diseases, if the recipient is counseled and her physician agrees to the insemination [11]. The general standard of practice in the industry, however, is to strongly urge recipients not to use such known donors. In all cases, the sperm bank must document with signed consent or risk acknowledgement that the recipient has been counseled and agrees to the potential risk to her and the offspring if an ineligible known donor is used for artificial insemination. This includes if a known donor is found to be carrier of a recessive genetic condition or there is a medical condition in the family history. With known donors, the 180-day quarantine and retesting are not required, but a risk acknowledgement must be signed by the recipient and partner (if applicable) [11, 12].

With both types of donors, the screening and testing process is basically the same. The American Society for Reproductive Medicine (ASRM) [21], the FDA [11], and state regulations (i.e., [10, 24, 25]) have regulations that must be followed by sperm banks providing donor sperm for clinical use. Both New York State [10] and California [24] require these facilities to apply for and be granted a state tissue bank license in order to operate in the state or ship donor sperm into the state. All states require FDA registration in order to operate in the USA.

Recruitment, Screening, and Testing of Sperm Donors

Sperm donors are often recruited by advertisement at local college campuses, as well as commercial online advertisers. Donors are also recruited through friends who may be currently participating as sperm donors. According to most guidelines, sperm donors should be of legal age, but generally less than 40 years of age [10, 20, 21] to minimize risks due to advanced paternal age. All applicants are required to fill out an extensive medical, family/genetic, and social history questionnaire. The purpose of this screening questionnaire is to determine any possible risks of genetic or medical conditions in the family history or ethnicity that could be passed on to offspring or increase the risk of a medical condition in offspring. The applicant's fertility history is documented, although past fertility, being beneficial, is not a requirement for participation. Equally important is the applicant's sexual and social history. Donors are screened for high-risk behaviors such as sexual orientation, multiple sexual partners, sexual activities in exchange for drugs or money, non-prescription drug use, tattoos, and body piercings, for example, to provide a better understanding of the potential donor's lifestyle. The medical history questionnaire is reviewed carefully by the sperm bank staff and medical director, and if acceptable, the applicant will have an in-person interview and thorough physical

examination to assess any physical evidence of high-risk behaviors. Applicants who have lived in or traveled to certain European countries for extended periods of time are rejected due to increased risk of variant Creutzfeldt–Jakob disease [11, 21]. A physical examination of the donor is performed to evaluate the donor based on criteria specified in FDA regulations, California, and New York requirements (e.g., tattoos, body piercings, evidence of high-risk behavior, and signs of HIV infection, genetic diseases) [10, 11]. The physical examination is part of the donor screening process in conjunction with genetic disease screening and serological testing for infectious diseases.

If the medical, genetic, and social histories are acceptable, the applicant will submit 3–5 specimens for qualification of acceptable sperm count, motility, and cryosurvival. These specimens are for testing purposes only and are thus not added to the inventory. If acceptable, the potential donor will have serological testing for evidence of infectious diseases [10, 11, 20, 21]. Donors are tested for HIV 1, 2 antibody screen, HTLV I/II antibody screen, hepatitis B (HBV) surface antigen and core antibody, hepatitis C virus, syphilis, *Neisseria gonorrhea*, cytomegalovirus (CMV) total antibodies (with reflex to IgG and IgM), *Chlamydia trachomatis*, and nucleic acid test (NAT) for HIV-1, HCV, and HBV. The blood type and Rh factor are also assessed and also hemoglobinopathy evaluation (including complete blood count (CBC) and screening for sickle cell anemia and thalassemia). Initial serological testing must be performed within 7 days of the first specimen [11] that will be placed into inventory.

Upon receipt, all test results are reviewed and recorded in the donor's file by 2 separate staff members. Positive results are submitted immediately to the medical director, who may determine whether the donor needs to be referred for counseling, retesting, or treatment. Positive results would make a person ineligible as an anonymous sperm donor. However, a positive CMV is not an automatic rejection. A donor can be accepted even with a positive CMV total antibody test. The sperm bank must have a specific policy regarding CMV status, including follow-up for IgG and IgM status, and subsequent routine testing for CMV total antibody. All anonymous donor sperm specimens must be held in quarantine for a minimum of 180 days, at which point the testing is repeated, and if negative, such specimens can be released for clinical use. Furthermore, anonymous donors are required to have a repeat physical examination and a repeat history questionnaire every 6 months while actively participating as a sperm donor [11]. In addition, donors fill out a risk factor questionnaire at each visit to determine any high-risk behaviors since previous participation [11]. If a donor is positive for *N. gonorrhea* or *Chlamydia*, he may reenter the program following treatment, a negative follow-up test, and a deferral period of at least 1 year. [11].

All anonymous sperm donors are tested for genetic conditions and carrier status as a prerequisite for participation. The genetic testing can be performed with initial serological testing, or prior to the first release of specimens for clinical use. Commercial genetic testing laboratories offer extensive panels for hundreds of recessive diseases, but not all are required by regulatory and professional agencies.

In fact, the FDA does not require genetic testing, being only concerned with communicable disease transmission. State regulatory agencies and professional associations (such as the ASRM) require genetic testing based on the donor's ethnic group [10, 21]. Genetic tests are required for carrier status for cystic fibrosis, Tay-Sachs disease, hemoglobinopathy/thalassemia/sickle cell anemia. Most sperm banks currently require the genetic testing for all donors regardless of reported ethnic group since mutations/deletions might become evident even if a certain ethnicity is far removed from the first- or second-degree blood relatives.

Genetic testing of sperm donors remains in the forefront of discussion by donor facilities and professional organizations in the USA and internationally. With genetic testing of sperm donors comes the issues of appropriate consenting of the donor for the genetic testing, limitations of the tests, potential implications for the donors' own children, and counseling of the donor in the case of positive carrier testing [26]. Even with extensive genetic testing, the risks for de novo mutations, multifactorial disorders, or undiagnosed disease cannot be reduced or eliminated with any genetic screening procedures [27–29]. There have been several lawsuits against commercial sperm banks and testing laboratories for incomplete testing as well as errors in testing.

Although US federal regulations do not address the issue of numbers of offspring per donor, standard of care requires that facilities establish a policy of number of offspring per donor, not including full blood siblings. The New York State regulations do not specify an acceptable number of offspring per donor [10], but the American Society for Reproductive Medicine suggests that there should be no more than 25 births in a population of 800,000 to avoid increased risk of consanguineous conception [21]. The Human Fertilisation and Embryology Authority (HFEA) in the UK limits the number of families a single donor can help to create at a limit of 10 [22].

It is essential to consent the donor in order to ensure that the applicant understands his responsibilities as a sperm donor, the confidentiality of participation, and including his denial of risk factors for communicable and genetic diseases [10, 21]. Furthermore, the donor must understand that his sperm is being used to create embryos that would be transferred to recipients in either heterosexual or same sex couples, single women, used for research, or discarded [11]. The FDA also requires that records pertain to donor screening and testing be maintained for at least 10 years [11, 21], while the New York State regulations require that records be maintained for 12 years if no pregnancy has occurred and 25 years where a pregnancy has occurred with that donor's sperm [10].

Client Depositor Sperm Banking

With major advances in medical treatments and high survival rates following cancer treatment, and advances in assisted reproduction, sperm banking is currently a routine procedure for male fertility preservation. Client depositors, often referred to

as “sperm bankers,” are those men who are banking their own sperm specimens for potential future use by a sexually intimate partner. The reason a man may elect to bank semen specimens includes prior to medical or surgical treatment for cancer, which may render him infertile or sterile, prior to vasectomy, prior to military deployment or potential workplace or environmental exposures, as well as prior to gender reassignment surgery [30–35]. Cryopreservation is also a routine procedure prior to assisted reproduction to avoid inconvenience due to failed semen collection often associated with stress. Prior cryopreservation is also helpful in the event of absence of the male partner at the time of the female partner’s estimated ovulation days [18, 20, 30]. Furthermore, testicular tissue and epididymal aspirates may be cryopreserved and used for multiple assisted reproduction cycles, thus avoiding repeated biopsies [1, 6–8, 30–32, 35]. Although experimental, prepubertal spermatogonial stem cell cryopreservation also is a promising possibility for fertility preservation allowing in vitro derivation of gametes from the stem cells [16, 17, 33–36]. Sperm banking and male fertility preservation should be an interdisciplinary approach, including oncologists, urologists, surgeons, reproductive endocrinologists, and mental health professionals, and a fully equipped laboratory with experienced staff.

Semen specimens can be collected by masturbation at the cryobank facility, or collected at home and delivered to the laboratory within 30–60 min of ejaculation. Although collection of donor semen is required on site at the laboratory, the client depositor may collect at home. This allows the possibility of a more comfortable and private environment, especially for young prepubertal men and hospitalized individuals. Some men may be unable to ejaculate by masturbation, due to psychological or medical reasons, and may be offered therapeutic options such as vibratory stimulation, electroejaculation, or surgically retrieved sperm [35]. Some men may not be able to ejaculate into a specimen container (i.e., antegrade ejaculation) and are afflicted with retrograde ejaculation due to surgical or drug-induced impairment of the ejaculatory process [35]. Protocols for alkalinization of the urine and recovery of viable sperm are available with subsequent cryopreservation of the retrograde sperm.

Client depositors or sperm bankers are expected to utilize their sperm specimens with a sexually intimate partner or spouse. However, in many instances, there may not be a partner identified at the time of sperm banking, or the banker might be a homosexual man. Furthermore, in the case of a minor child, sperm banking might be requested by a parent.

Detailed and complete consenting is required for the client depositor desiring fertility preservation. The cryobank must obtain written consent from the client depositor prior to storing semen, and, after all details of sperm banking have been presented to the client. The details include the procedure for retrieval, collection, and storage of the sperm, risks of drugs and surgical procedures, as well as payment for processing and storage [10]. Furthermore, the consent must also indicate specific instructions on assignment or disposition of the frozen semen upon the client’s death or if he is rendered incapable of making conscious decisions or is rendered mentally incompetent [10]. The choices available include discard or

transfer of the specimens to the control of a designated person who is then responsible for complying with the elements of the client's consent. The cryobank must have written procedures to ensure that these directives are followed.

As with the consenting of a known or directed donor, if a client depositor may want to designate the sperm to a non-sexually intimate partner, he must be screened and tested according to the FDA regulations [11] and applicable state regulations [10]. Thus, a client depositor may then become a directed donor and must undergo the screening and testing that is required. Furthermore, if risk factors are identified, or communicable disease test results are positive, risk waivers must be signed by any recipient of the specimens. Thus, if there is any possibility that a client's sperm specimens might be used by a non-sexually intimate partner, he should be urged to undergo the screening and testing process. This certainly increases the cost to the patient and more than likely the stress at a particularly difficult time. But it is the responsibility of the cryobank to insure that the client is informed and prepared for future possibilities, including not being able to use the specimens in the future because regulations were not followed.

Clinical use of the sperm specimens is under the direction of the health professional caring for the recipient. Often, the recipient may be under the care of a fertility or assisted reproductive technology (ART) specialist, especially if the original banked specimens are suboptimal. The cryobank must secure consent from the sperm banker to transfer his specimens to a facility for the use of the named recipient. At that time, specific information is provided to the health professional, including specimen information (date of banking, specimen post-thaw parameters) as well as any screening and testing results. Again, screening and testing is not required if the specimens are used in a sexually intimate partner, but if not used in a sexually intimate partner, a donor eligibility determination is required.

Semen Cryopreservation

Successful cooling, freezing, storage, and thawing of human semen specimens with subsequent maintenance of cell viability and clinical utility require not only a basic understanding of principles of cryobiology, but a facility and laboratory staff capable of integrating all aspects of the laboratory and clinical sciences [20, 37]. Cryoprotective agents (CPAs) are used with various protocols to protect the cells from intracellular ice formation and osmotic shock during freezing and thawing [1, 3, 4, 20]. The most commonly used cryopreservative for sperm freezing is glycerol at a concentration of 5–10%, either alone or with extenders such as a buffer or protein such as hen's egg yolk [3–5, 20]. Currently, most cryobanks use glycerol in hen's egg yolk; i.e., TEST–yolk–glycerol freezing medium (Irvine Scientific, Irvine, CA), or similar commercially available cryopreservative solutions.

Following slow mixing of the CPA with the semen specimen to avoid osmotic shock, the semen–CPA mixture is placed into a labeled plastic cryovials or secure plastic straws at a volume of 0.5 ml [5, 20, 30, 37]. Cryovials have been the preferred vessel for semen since the vials provide a larger surface-area-to-volume ratio for uniform sample cooling, are able to withstand ultra-low temperatures without breakage, are easily manipulated, and have ample space for clear labeling [37]. The inventory system is also more easily organized and manipulated in storage tanks.

The method for freezing involves cooling using either a slow or rapid method. As a rule of thumb, the thaw rate should match the freeze rate [1, 20, 37]. The specimens can be frozen using either a programmable freezer or manual freezing. A programmable or controlled rate freezer slowly reduces the temperature of the specimen starting with room temperature, followed by a cooling rate of −50 °C per minute to 4 °C and then at a rate of −10 °C per minute down to −80 °C. The specimens are held at −80 °C for 10 min and then plunged into liquid nitrogen (LN) [5]. For manual freezing, the vials may be cooled in a refrigerator for 30–45 min and then slowly acclimatized to liquid nitrogen temperature by suspending the vials in vapor (approximately −75 °C) for 10 min. The vapor phase exposure is followed by plunging into the liquid nitrogen [5, 30]. Alternately, the vials, affixed to a metal cane, can be placed directly into an LN-charged dry shipper for 30 min and then plunged into the liquid phase in a tank for storage [5]. It is good laboratory practice to validate the actual procedure used by testing the CPA and freeze protocol with donated sperm specimens to ensure the best possible cryosurvival. A test vial (or straw) containing at least 0.3 ml of semen–CPA should be thawed 24–48 h after the initial freeze to determine the post-thaw motility and hence cryosurvival. This information is provided to the client depositor and his healthcare practitioner so that appropriate decisions can be made for further processing of the specimen for insemination or ART. As with any client specimen, the availability of the post-thaw test vial is dependent on the initial semen volume. A test vial might not be available for low-volume specimens as it is more advantageous to freeze for future clinical use than to test thaw for report purposes.

Semen can be cryopreserved as neat, whole semen using the CPA and methods indicated previously for use with cervical insemination. This is referred to as an "ICI" or intracervical vial. However, semen can also be processed by gradient centrifugation or simple wash-centrifugation procedure to prepare an "IUI-ready," or intrauterine insemination-ready, vial [5]. The sperm can be washed free of seminal fluid constituents and motile sperm recovered using the laboratory's standard method, using commercially available nutrient media such as HEPES-buffered human tubal fluid or Ham's F-10 medium. Following gradient or simple centrifugation, the pellet is re-suspended in the medium, and the CPA is added to a final concentration of 7.5–8.0% glycerol [5]. Preference for ICI- or IUI-prepared vials is dependent on the end user practitioner's needs and facility. Most specialized laboratories prefer to process the ICI specimens in their own

laboratory in preparation for insemination or IVF/ICSI. Private practitioners may not have this capability and would therefore prefer "IUI" or "washed" sperm, ready for an intrauterine insemination immediately upon thaw.

Quality control in the sperm bank is a primary concern not only to comply with regulatory agencies, but also to provide the patient and client with the optimal quality of care. General laboratory quality control-checking and recording of equipment and room temperatures, equipment preventative maintenance, cell counting quality control, as well as reagent and process validation are essential within the laboratory. Exact attention to and verification of specimen labeling and record-keeping processes are essential parts of good laboratory procedure [37]. Detailed written quality control and quality assurance manuals are an important reference in the sperm bank laboratory.

Of particular note is the importance of labeling and specimen tracking [5, 37]. It is essential that the vials be labeled with more than 1 identifier. Usually, this is the client depositor name and either date of birth or a unique accession number or patient identifier. The vial should also be labeled with the date of the specimen as well as the name or initials of the cryobank that performed the specimen freezing and storage. Vials of anonymous donor semen are labeled with the donor code number, the date of the specimen, and the cryobank name or initials. Directed donor specimen vials are also labeled with a code number specific to that donor or the donor's name and date of cryopreservation. A written or computerized log of the location of all vials, with information on distribution of each vial, allows the tracking of each and every vial for clinical use. Quality laboratory practice also includes double verification of vial identity upon removal from a tank in preparation for shipment.

Storage in liquid nitrogen for long-term storage of semen specimens is preferable than vapor storage [37], although vapor storage may be advantageous especially for clients who have tested positive for communicable diseases. There is no evidence of transmission of disease in an LN tank [38]. However, it is recommended that client depositor specimens are kept in separate tanks than donor specimens [10].

Tanks should be closely monitored, either on a daily basis or at a minimum of 3 separate days in a 7-day period of time [5, 10, 37]. Liquid nitrogen levels are manually measured using a measuring stick, and tanks are filled with liquid nitrogen when the liquid level decreases lower than a preset level. Certain tanks are more sensitive to malfunction, especially the automatic fill tanks where electrical solenoid fixtures monitor liquid level and turn on auto-fill from a large LN dewar. The solenoid can malfunction if repeatedly exposed directly to the LN temperatures. Tank alarms may also be installed that notify staff by way of a beeper, automated phone call or warning system if the LN level reaches low preset level. Staff members can then answer the call, appear in person at the laboratory, or may remotely diagnose or correct a system fault. [37]

Management and Infrastructure of the Cryobank

The need for facilities to provide male fertility preservation has increased over the last 60+ years with the realization of increased survival rates following medical treatments and as the scientific community has developed improved methods for gamete cryopreservation and storage. As with any business or commercial entity, a sperm bank requires a strong executive management structure, as well as sales and marketing divisions and, of course, the laboratory providing the semen cryopreservation and storage. Since the sperm bank performs semen analyses on both donors and client depositors, the laboratory is considered a clinical laboratory and therefore is subject to the rules and regulations governing clinical laboratories. The Centers for Medicare and Medicaid Services (CMS) regulates all clinical laboratory testing performed on human specimens in the USA through the Clinical Laboratory Improvement Amendment (CLIA 1988) [39]. State regulations and state licensing may also apply. As required by federal and state regulatory agencies, sperm banks fall under the same requirements as other tissue banks.

Laboratory Director

The company owner must enlist a qualified and board-certified laboratory director who meets criteria for experience as well as regulatory compliance. The laboratory director must have a Ph.D. or MD degree with board certification by a recognized organization.

The laboratory director is responsible for the overall operation and administration of the laboratory, including the employment of personnel who are competent to perform test procedures, record, and report test results promptly, accurately, and proficiently, and for assuring compliance with the applicable regulations [39]. The laboratory director may also perform the duties of a technical supervisor, general supervisor, or clinical consultant and perform laboratory testing.

Medical Director

A sperm bank must also retain a medical director who is responsible for the medical aspects of the sperm bank in consultation with a medical advisory committee. These responsibilities include monitoring the efficacy of the program, including medical criteria for donor participation, quality standards, guidelines for the number of pregnancies per anonymous sperm donor, and selection of donors who meet the medical criteria of the sperm bank [10, 21]. The medical director would also review and implement consenting of donors, donor interviews, and donor physical examinations, as well as review reports of adverse reactions and/or genetic conditions from inseminations.

Tissue Bank Director

A tissue bank director may also be appointed in addition to the medical and laboratory directors. The tissue bank director is responsible for overall operation of the sperm bank and shall develop and implement policies and procedures of the sperm bank [10]. Furthermore, the tissue bank director is also responsible for regulatory compliance.

Technical Staff

The technical staff of the sperm bank must be qualified and trained according to the laboratory's standard operating procedures. Similarly, in the USA, there are federal and state requirements for the testing personnel of a laboratory and consequently the sperm bank [10, 39]. Significant responsibility is given to the technical staff for processing and storage of semen specimens and for laboratory quality control, especially specimen labeling and storage tank oversight.

Licensing

In the USA, all tissue banks must be registered with the FDA under 21CFR 1271 [11]. New York, [10] California, [24] and Maryland [25] require a separate state tissue bank license if specimens are to be shipped into that state. Each state requires a lengthy application process. Standard operating procedures must comply with the individual state regulations. Furthermore, California requires client depositors to be tested for communicable diseases either at the time of initial sperm banking or before transfer of specimens into California [25].

Conclusion

There is much that goes into establishment, organization, and operation of a sperm bank. These include policies and procedures, management and staffing, and licensing and accreditation. The larger facilities employ staff specifically devoted to regulatory compliance in addition to medical and tissue bank directors. A significant financial investment is required for the facility, personnel, sales, and marketing.

Clinical Vignettes

These vignettes are meant to stimulate thinking and discussion in relation to the regulations, standard of care, and what you might set as policy in your practice.

1. Mr. Smith requests to bank semen specimens for possible future use. He states that his female partner is not able to carry a pregnancy, and therefore, they plan to use her oocytes, create embryos with his sperm, and transfer the embryos to a gestational carrier, his partner's sister.

   a. Is risk factor screening and communicable disease testing required? If so, briefly summarize the process.

      Discussion: In this situation, both Mr. Smith and his female partner are considered directed donors to the gestational carrier. According to the US FDA regulations, both must undergo donor eligibility determination and communicable disease testing. Since the gestational carrier would be known to Mr. Smith and his partner, if risk factors are identified, or test results are positive for communicable disease, the gametes/embryos can still be used as long as the gestational carrier is informed, counseled, and consents to receive the embryos.

2. A student responds to an advertisement for sperm donors in his college newspaper by calling the local sperm bank.

   a. What would be an appropriate age range for the sperm donors?

      Discussion: Generally, the minimum age of sperm donors is 18 years of age, although sperm banks may choose to use 21 years of age as the minimum age of the donors. The maximum age of anonymous sperm donors is recommended as 40 years of age, due to the increased risk of DNA fragmentation, and possible genetic defects with increasing paternal age.

   b. Is there a minimum height for sperm donors? Do you think that there should be a minimum height and weight for sperm donors?
   c. The college student states that he is not homosexual, but did engage in some sexual interaction with a male once when inebriated. Is this a potential risk factor? Would you allow this person to be screened and accepted as an anonymous sperm donor?

3. The sperm bank receives a call from a recipient of donor sperm reporting a pregnancy with anonymous donor sperm from that sperm bank. The child, however, has been diagnosed with congenital adrenal hyperplasia. The child is doing well, but is closely monitored by the pediatrician.

   a. How would the sperm bank investigate this occurrence?

      Discussion: The sperm bank should consult with a genetics counselor who will provide information on this condition and the potential for genetic

inheritance. There may currently be genetic testing of the donor, mother (or egg donor), and/or the child. Assuming there is no family history of genetic conditions, is the sperm bank liable or legally responsible for this outcome?

b. Should anyone who has purchased this donor's specimens be notified? Should the donor be notified?

Discussion: It is prudent to notify anyone who purchased this donor's specimens of the occurrence of this genetic condition. The condition and potential that any offspring would be a carrier should be discussed. The donor should also be notified so that he can make informed decisions regarding his own family planning.

c. Should the donor's specimens still be provided for sale by the sperm bank? If so, should potential recipients be informed of the genetic condition?

4. A couple requests use of a directed or known sperm donor specimens for insemination of the female partner. The directed donor is the homosexual brother of the male partner. The donor's partner is HIV positive.

   a. Would you agree to screen this donor and cryopreserve his specimens to be used with this recipient?

5. The local hospital contacts the sperm bank to request sperm banking for a patient who started chemotherapy 5 days ago.

   a. Would the recent chemotherapy be expected to affect the sperm concentration and motility? DNA fragmentation? Is there a risk to potential offspring?
   b. Should sperm banking be offered to this patient?
   c. What are the recommendations for fertility preservation if this patient is 16 years of age, with no current sexual partner or future plans for fertility? Consider use of the sperm in a future non-sexually intimate partner; if the patient die, who would have control of the specimens?

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Chapter 16
Assisted Conception Techniques: Which One to Choose

Pankaj Talwar and Ashish Fauzdar

Introduction

Infertility is a disease of reproductive system defined by failure to achieve the clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. Infertility or subfertility affects 8–12% of couples worldwide of which 40–50% is due to "male factor," either solely (20%) or in combination with the female factor (30–40%) [2, 3]. In another 30–40% of cases, no male-infertility-associated factor is found (idiopathic male infertility) [4]. Male fertility can be reduced as a result of congenital or acquired urogenital abnormalities, malignancies, or urogenital tract infections. These abnormalities may lead to increased scrotal temperature (e.g., as a consequence of varicocele), endocrine disturbances, genetic abnormalities, and immunological changes. All these etiologies influence will influence negatively one or more of sperm quality parameters of sperm concentration, sperm motility, or percentage normal morphology. In addition to the standard medical and surgical infertility treatments for male infertility assisted reproductive technologies, or ART other an additional therapeutic modality. The most common ART procedure is in vitro fertilization (IVF), but there are new procedures that are continuously evolving to enhance the IVF success rate and address infertility-related issues. It is also important to investigate all men

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undergoing infertility treatment with a thorough history and physical examination before any clinical intervention is planned with ART. This will also allow patients with irreversible infertility to be separated from those with potentially treatable

Table 16.1 Basic investigation of the infertile man

Type of investigation	Area of focus	Clinical utility of investigation
Urinalysis	Urine sample	To indicate the presence of an infection
Semen analysis	Semen volume, sperm concentration, motility, morphology, and sperm antibodies as per WHO 2010 Edition [5]	The evaluation assesses sperm motility or movement, the shape and maturity of the sperm, the volume of the ejaculate, the actual sperm count, and the liquidity of the ejaculate
Hormonal measurements	Luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, testosterone, sex-hormone-binding globulin, thyroid-stimulating hormone (TSH)	Determine the overall balance of the hormonal system and specific state of sperm production To be carried out when we have a patient with no or very low sperm count Clinical examination reveals testicular failure
Imaging	Scrotal ultrasound, transrectal ultrasound of prostate and seminal vesicles, and magnetic resonance imaging of pituitary and genitourinary tract	To detect varicocelesor obstruction of the conducting system These diagnostic modalities should not be performed until indicated, as doing them routinely leads to the escalation in patient expenditure **There is no substitute for thorough clinical examination of the male genital system**
Testis Biopsy	Needle aspiration: cells or tissue	To determine if sperm production is impaired or a blockage exists Freeze the spermatozoa extracted for further utilization **No role of diagnostic biopsy** Procedure should be done at assisted reproduction technologies (ART)/ urology center with the aim of cryo-freezing the sample if spermatozoa are identified on microscopic examination of the tested testicular tissue sample
Semen culture	Infection in the ejaculated sample—as per WHO 2010 guidelines [5]	Can be carried out routinely, but ideally done in all cases when we diagnose significant pus cells in the ejaculated sample

conditions or subfertility, usually by standard clinical evaluation and physical examination and with some basic investigations as per their clinical utility (Table 16.1) [5].

Common Andrological Challenges (Semen Parameters)

Male infertility can be due to any of the 3 broad categories: ejaculation or erection problems (coital disorders), problems with small reproductive tract ducts with obstruction in ejaculatory duct, or problems with sperm production. The problem with sperm production is the most common of the 3 and can be detected during semen analysis. Semen analysis is still the cornerstone to investigate male infertility and remains the single most useful and fundamental investigation, with a sensitivity of 89.6%, to detect genuine problems of male infertility (Figs. 16.1 and 16.2) [6]. As we know, the sperm analysis does not provide insights into the functional potential of the spermatozoon and subsequent maturation processes required to achieve fertilization. The World Health Organization (WHO) has revised lower reference limits for semen analyses. Table 16.2 shows the parameters representing the accepted 5th percentile (lower reference limits and 95% confidence intervals [CIs] in parentheses) [5].

Fig. 16.1 MAKLER® counting chamber for rapid sperm analysis for counting spermatozoa. (Manufacture by SEFI Medical Instruments Ltd. Distributed by: Irvine Scientific 2511 Daimler St. Santa Ana, CA 92705)

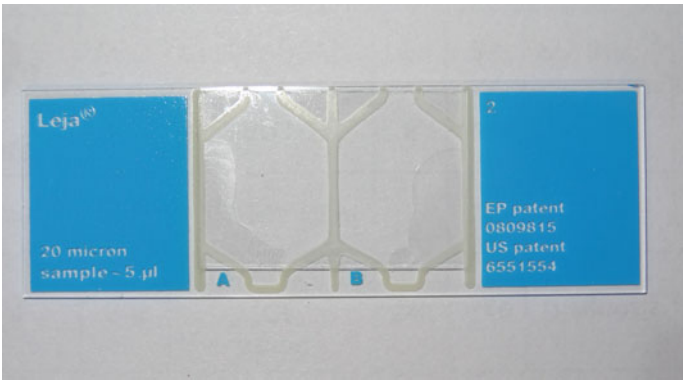

Fig. 16.2 Leja® slides are high-quality disposable counting chambers for semen analysis with increased level of accuracy and precision. Manufactured by Leja Luzernestraat, 10 2153 GN Nieuw-Vennep, The Netherlands

Table 16.2 Lower reference limits (5th percentiles and their 95% CIs) for semen characteristics

Parameter	Lower reference limit (range)
Semen volume (mL)	1.5 (1.4–1.7)
Total sperm number (10^6/ejaculate)	39 (33–46)
Sperm concentration (10^6/mL)	15 (12–16)
Total motility (PR + NP, %)	40 (38–42)
Progressive motility (PR, %)	32 (31–34)
Vitality (live spermatozoa, %)	58 (55–63)
Sperm morphology (normal forms, %)	4 (3.0–4.0)
Round cell concentration	Less than 1×10^6 per ml

Adapted with permission from WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th edition. 2010. Appendix 1. Table A1.1, page 224, http://apps.who.int/iris/bitstream/10665/44261/1/9789241547789_eng.pdf

CIs = confidence intervals; NP = non-progressive; PR = progressive

As per our present understanding, males with sperm parameters below the WHO normal values are considered to have male factor infertility [6]. The most significant of these are no spermatozoa in the ejaculate (azoospermia) (Fig. 16.3), low sperm concentration (oligozoospermia), poor sperm motility (asthenozoospermia, <32% progressive motile spermatozoa), and abnormal sperm morphology (teratozoospermia <4% normal forms). Often, all 3 anomalies occur simultaneously, which is defined as oligo-astheno-teratozoospermia or OAT syndrome. It has been observed that as high as 90% of male infertility problems are related to sperm concentration in ejaculate, and there is a positive association with abnormal semen parameters and sperm count [7]. The problem with sperm count, motility, and morphology can arise from disorders in control mechanism, including pre-testicular, testicular, and post-testicular factors [8].

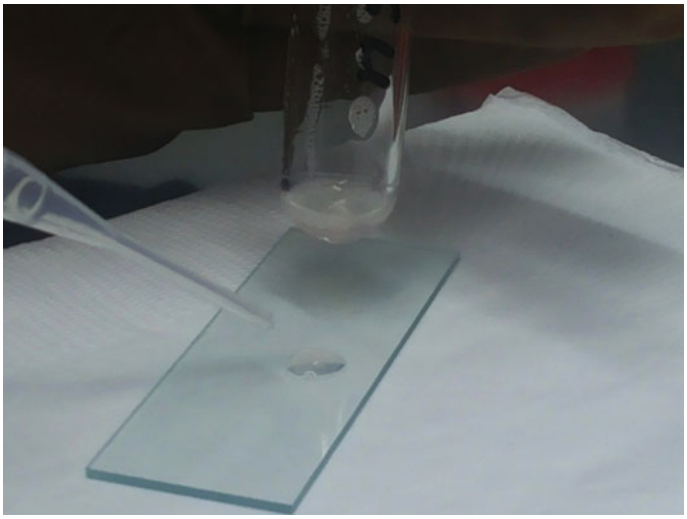

Fig. 16.3 Diagnosis of azoospermia by observing spermatozoa in the sediment of a centrifuged sample

The common andrological challenges that occur during semen analysis of patients with infertility due to male factor, and clinical interventions to help these patients achieve fertility, are summarized in Table 16.3 (see also Fig. 16.4).

Obstructive Azoospermia

Obstructive azoospermia (OA) is the absence of spermatozoa and spermatogenetic cells in semen and post-ejaculate urine due to obstruction (Fig. 16.5). OA is less common than non-obstructive azoospermia (NOA) and occurs in 15–20% of men with azoospermia. Men with OA present with normal follicle-stimulating hormone (FSH), normal size testes, and epididymal enlargement.

Obstruction in primary infertile men is often present at the epididymal level. Epididymal obstruction is the most common cause of OA, affecting 30–67% of azoospermic men. Congenital epididymal obstruction usually manifests as congenital bilateral absence of the vas deferens (CBAVD), which is associated with at least 1 mutation of the CF gene in 82% of cases. Intratesticular obstruction occurs in 15% of men with OA. Ejaculatory duct obstruction is found in 1–3% of cases of OA and is classified as either cystic or post-inflammatory [9, 10].

Table 16.3 Common andrological challenges (semen parameters) while treating patients of male infertility

Basic investigation	Provisional diagnosis	Clinical observations (semen parameters)	Additional investigation (enhanced diagnostics evaluation)	Assisted reproductive technology—to choose	Clinical intervention
Semen analysis	**Normal**	Normal semen parameters as per WHO Guidelines, 2010 [5]	Not required	**Sample preparation technique**: Density Gradient/: Swim-up Techniques	Insemination: IUI/IVF
Abnormal	1. **Low sperm counts** *Oligozoospermia*	a. SEVERE oligospermia ($\leq$1 million/ml)	Confirm by repeating the semen analysis if abnormal in at least 2 tests as per WHO Guidelines, 2010 [5] Semen culture for bacterial infection Y-chromosome micro deletion Peripheral karyotype ROS estimation Leukocyte detection test when round cells count is >1 $\times$ 10^6	Surgical sperm retrieval if sperm is with poor motility and/or does not survive freez-thaw cycle $\rightarrow$ ICSI Otherwise fresh prepared sperm is used. Consider sperm cryobanking to avoid unexpected absent sperm on the day of egg collection When the ejaculated sample is grossly infected, necrospermia exists, do appropriate DNA fragmentation studies, ROS and vitality analysis studies before proceeding	- If positive ROS test with coexisting bacterial infection with presence of pus cells, start on **Anti-oxidant (AOX) therapy**, and **Antibiotic treatment** $\times$ 3–6 months $\downarrow$ Repeat semen analysis after 3 months $\downarrow$ IVF/ICSI (Depending on sperm count/motility) - If handling a case of idiopathic OAT administer AOX for 3–6 months before intervention - Doing SSR is an option in still unsatisfactory samples
		b. $\leq$5 million/ml		b and c ICSI from ejaculated sample	
		c. $\leq$10 million/ml			
	2. **Poor sperm motility** *Asthenozoospermia*	$\leq$32% progressive motile	Vitality staining (eosin-nigrosin or plain wet preparation with eosin only) Hypo-osmotic swelling (HOS) test	ICSI along with HOS test or use of theophylline/pentoxifylline depending on the laboratory practice	Start on **Anti-oxidant therapy**, and **Antibiotic treatment** X 3 months. $\downarrow$ IVF/ICSI if no improvement or minimal benefit observed.
	3. **Abnormal sperm morphology** *Teratozoospermia*	$\leq$4% normal forms	Sperm morphology assessment by using pre-stained slides or conventional staining of the sample mounted slides	IMSI may benefit ICSI with selection of good sperms TESE $\rightarrow$ ICSI is controversial	IMSI is treatment of choice for these patients
	4. **Reduced vitality**	$\leq$58% live spermatozoa	Consider HOS test to assesses the functional	ICSI using cryo HOS or sperm Mobil	Start on **Anti-oxidant therapy**, and **Antibiotic treatment** $\times$ 3–6 months

(continued)

Table 16.3 (continued)

			integrity of the human sperm membrane and sperm vitality Eosin or eosin-nigrosin staining		ICSI
	5. **Oligoasthenoteratozoospermia (OAT) syndrome**	Abnormal sperm count, motility, and morphology	Semi-quantitative assessment of DNA by DNA Fragmentation Index (DFI) by Sperm Halo test	ICSI or TESE → ICSI	Anti-oxidants therapy, repeat DFI after 3–6 months, followed by ICSI
	6. **No sperms** *Azoospermia*	Obstructive azoospermia (OA)	Hormone analysis (FSH, LH, and testosterone) Transrectal ultrasound (TRUS) to detect obstruction in ejaculatory duct (not mandatory) Magnetic resonance imaging (MRI) (not mandatory) Cystic fibrosis gene mutation (CF) analysis	ICSI following SSR (PESA/MESA) is treatment of choice for OA patients with epididymal blockage or absence or inflamed vas deferens	Vasal aspiration, microsurgical or percutaneous epididymal sperm aspiration (MESA/PESA), → Cryopreservation of sperms → ICSI (later IVF cycle) If no sperms-TESE/TESA
		Non-obstructive azoospermia (NOA) Testicular failure (hypergonadotropic hypogonadism) Hypogonadotrophic hypogonadism	Hormone analysis FSH, LH and testosterone Testicular biopsy (with normal FSH and testicular volume) Y-chromosome microdeletion for AZFc gene deletion status Karyotype of male	Testicular sperm retrieval and ICSI are treatment of choice for NOA patient with testicular failure.	Rule out genetic diseases Treat hypogonadotrophic hypogonadism with HMG (human menopausal gonadotropin) and HCG (human chorionic gonadotrophin) for 6 months to 1 year before sperms are detected in the ejaculate If AZFc deletion: proceed to sperm retrieval through TESE → IVF/ICSI If AZFa + AZFb deletion: TESE technique is not recommended here as the procedure is unlikely to harvest sperms for ICSI Adequate counseling is essential

WHO—World Health Organization, IUI—intrauterine insemination, IVF—in vitro fertilization, HOS—hypo-osmotic swelling, ICSI—intracytoplasmic sperm injection, ROS—reactive oxygen species, OAT—oligo-astheno-teratozoospermia, SSR—surgical sperm retrieval, IMSI—intracytoplasmic morphologically selected sperm injection, TESE—testicular sperm extraction, DFI—DNA fragmentation index, FSH—follicle-stimulating hormone, LH—luteinizing hormone

Non-obstructive Azoospermia

Non-obstructive azoospermia (NOA), also referred to as testicular failure, occurs in 49–93% of patients with normal semen ejaculate volume with no sperms (Fig. 16.6). The testicular failure indicates a complete absence of spermatogenesis, but men with testicular failure actually have also either reduced spermatogenesis, maturation arrest, or complete failure of spermatogenesis noted with Sertoli-cell-only syndrome [11, 12]. In men with testicular deficiency, hypergonadotropic hypogonadism is usually present, with high levels of FSH and luteinizing hormone (LH), and sometimes low LH and FSH levels with low or normal levels of testosterone. The levels of FSH correlate with the number of spermatogonia; when spermatogonia are absent or markedly diminished, FSH values are usually elevated. However, for an individual patient, FSH levels do not accurately predict the spermatogenesis status because men with maturation arrest histology could have normal FSH and normal testis volume and still be azoospermic. Testicular biopsy is usually the method of choice to provide definitive diagnosis and also as a part of the treatment option [13–15].

Y-Chromosome Microdeletion

Indications for AZF deletion screening are based on sperm count and include the patient presenting with azoospermia and severe oligozoospermia (spermatozoa count <5 million/mL). The highest frequency of Y-deletions is found in azoospermic men (8–12%), followed by oligozoospermic (3–7%) men [16]. The microdeletion of the Yq chromosome is not indicated in normozoospermic men, proving there is a clear-cut cause-and-effect relationship between Y-deletions and spermatogenic failure [17]. AZFc deletions are most common (65–70%), followed by Y-deletions of the AZFb and AZFb + c or AZFa + b + c regions (25–30%). AZFa region deletions are rare (5%). Complete removal of the AZFa region is associated with severe testicular phenotype (Sertoli-cell-only syndrome), while complete removal of the AZFb region is associated with spermatogenic rest. Complete removal of the AZFc region causes a variable phenotype ranging from azoospermia to oligozoospermia. The specificity and genotype/phenotype correlation reported above means that Y-deletion analysis has both a diagnostic and prognostic value for testicular sperm retrieval [18]. The common genetic investigations required in the workup of infertile patient due to “male factor” with their relative frequency are summarized in Table 16.4.

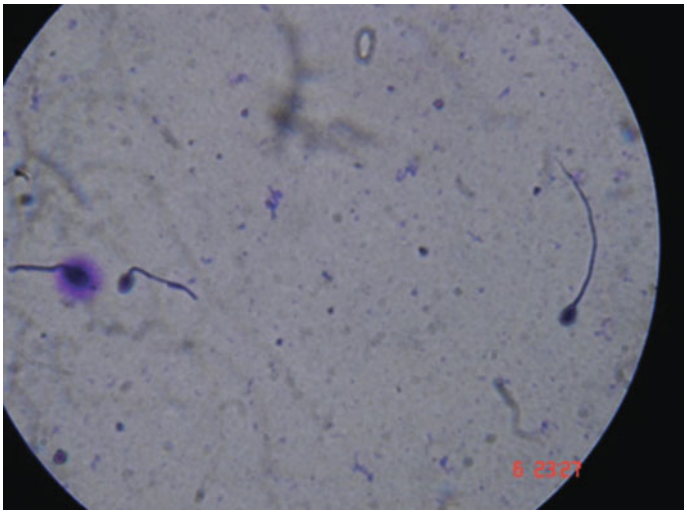

Fig. 16.4 DNA fragmentation test. Cells showing *halo* around the nucleus are good sperms

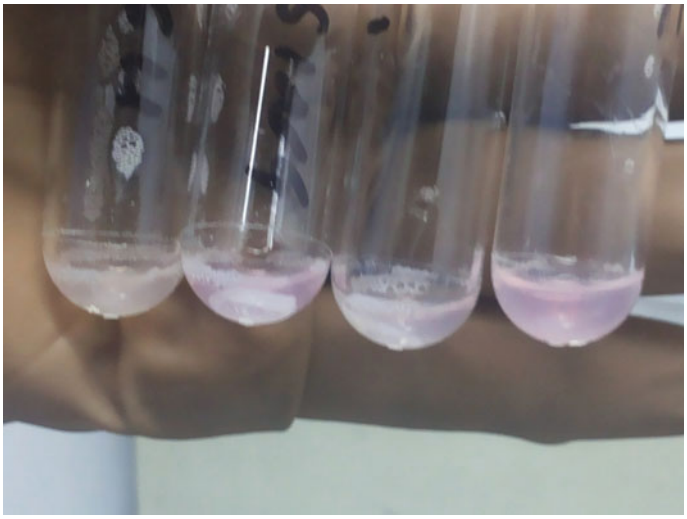

Fig. 16.5 Sperm retrieval and sample preparation from patient with retrograde ejaculation

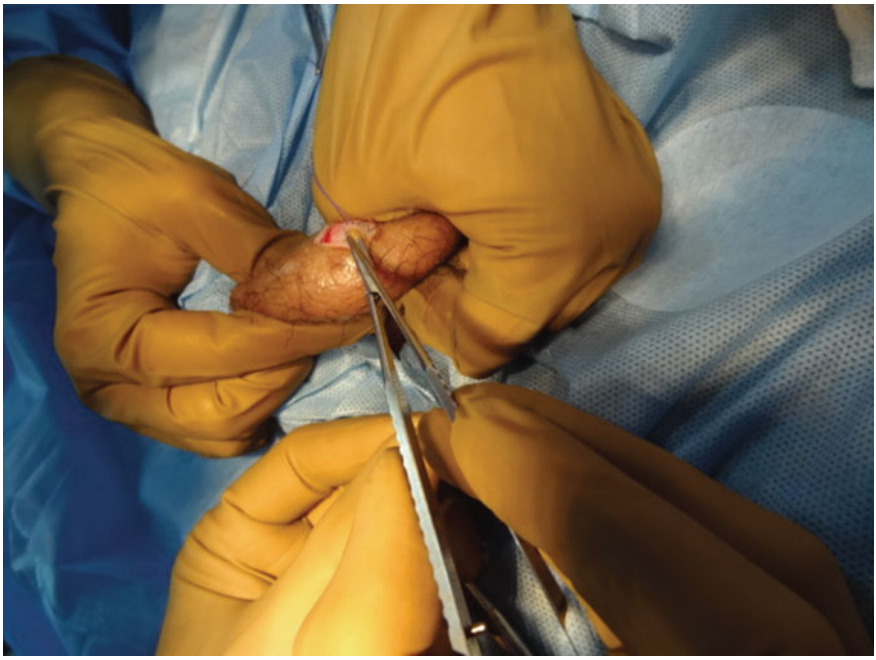

Fig. 16.6 Procedure for testicular sperm extraction from non-obstructive azoospermic (NOA) patients

Common Clinical Challenges

Coital Disorders

The most common male coital disorders impacting fertility include erectile dysfunction (impotence), failure of ejaculation (anejeculation), and retrograde ejaculation. These disorders are more common in patients with neurologic diseases and patients with—who desire fertility. These patients face challenges that include hormonal imbalances, ejaculatory dysfunction, and poor semen quality. The list of common clinical challenges encountered in patients with male factors, and their subsequent management, is summarized in Table 16.5 (see also Fig. 16.7).

Erectile Dysfunction (ED) is defined as the inability to achieve and/or maintain penile erection sufficient for satisfactory sexual performance [19]. It also is associated with low libido due to androgen deficiency with primary or secondary hypogonadism. Erectile dysfunction that is related to vascular or neurologic abnormalities (diabetic autonomic neuropathy or pelvic nerve damage) is uncommon in men presenting with infertility [20].

Anejaculation is defined as the absence of either antegrade or retrograde ejaculation, and this is common in patients with spinal cord injury (SCI). Overall, only

Table 16.4 Common genetic investigation in an infertile male

Clinical indications	Genetic investigation (% frequency)		
	Cystic fibrosis	Karyotype	Y-chromosome microdeletion
1. Obstruction azoospermia (OA) with absence of epididymal, seminal vesicles or ejaculatory duct congenital absence of vas deferens (CBAVD)	✓	✕	✕
2. Testicular failure (non-obstructive azoospermia)	✕	✓	✓
3. Oligozoospermia (≤ 10 million/ml)	✕	✓	✕
4. Oligozoospermia (≤ 5 million/ml)	✕	✓	✓
5. Family history of recurrent spontaneous abortions, malformations, or mental retardation	✕	✓	✕

9–15% of men with SCI are able to ejaculate in response to normal stimuli [21]. This may also be caused by antihypertensive and psychotropic drugs and is uncommon cause of infertility in most societies [22].

Retrograde Ejaculation is defined as failure of closure of the urinary bladder neck, and this results in reflux of semen into the bladder (Fig. 16.5). Clinically, this manifests with a lower volume of semen ejaculate and sperms identified in the post-ejaculatory urine [23]. Retrograde ejaculation can occur as a result of SCI, neuropathy due to diabetes or other neurologic conditions including multiple sclerosis (MS), or surgical interventions such as a retroperitoneal lymph node dissection (RPLND).

Varicocele

Varicocele is a variation of normal anatomy with enlarged veins in the scrotum. This may be associated with andrological conditions such as failure of ipsilateral testicular growth and development, with symptoms of pain and discomfort, male subfertility, and hypogonadism. Varicocele is a physical abnormality that is present in 11.7% of adult men and in 25.4% of men who have abnormal semen parameters [24]. The exact association between reduced male fertility and varicocele is unknown, but recent studies have shown that semen improvement is usually observed after surgical correction [25].

Cystic Fibrosis

Cystic fibrosis disease is associated with congenital bilateral absence of vas deferens (CBAVD) with absence of vas deferens and/or seminal vesicles. These patients have a mutation in the CFTR gene that encodes a membrane protein that

Table 16.5 Common clinical challenges we face while treating patients of male infertility

Clinical challenges	Common disorders	Clinical observations	Additional diagnostic workup	Assisted reproductive technology—to choose	**Clinical Intervention**
Coital disorders	*Anejaculation*	Complete absence of antegrade or retrograde ejaculation due to spinal cord injury	Cystoscopy transrectal ultrasound (TRUS)	Vibrostimulation, electro ejaculation, or surgical sperm retrieval Testicular (TESE or PESA) or epididymal (MESA) → sperm preparation → ICSI	Insemination technique → IUI/IVF/ICSI depends on sperm retrieval and quality (concentration and motility) in the post-wash sample
	Erectile dysfunction	Inability to achieve and/or maintain penile erection and orgasm sufficient for semen ejaculation			
	Retrograde ejaculation	Absence of antegrade ejaculation and semen passing to urinary bladder due to failure of bladder neck to close with orgasm	Post-ejaculate urine for the presence of sperms	Biological sperm collection from post-orgasmic urine → sperm preparation → IUI/IVF	
Varicocele	Variation of normal anatomy—veins in the scrotum enlarge and give worm in bag appearance	Abnormal semen analysis with reduction in the sperm count and/or sperm motility	Physical examination and grading of varicocele	Surgery with incision in the groin and spermatic cord containing vas deferens (this has indications and should not be advised in all cases) IUI/IVF or ICSI	Insemination technique → IUI/IVF or ICSI depending on the sperm quality (concentration and motility)
Cystic fibrosis	Absence of vas deferens	Absence of vas deferens and/or seminal vesicles. Congenital bilateral absence of vas deferens (CBAVD)	1. Transrectal ultrasound (TRUS) 2. Cystic fibrosis (CFTR) gene mutation testing of both partners	Genetic counseling Sperm retrieval MESA/PESA → ICSI	Thorough counseling PESA/ICSI Donor insemination PGD

TESE—testicular sperm extraction, PESA—percutaneous epididymal sperm aspiration, MESA—microsurgical epididymal sperm aspiration, ICSI—intracytoplasmic sperm injection, IUI—intrauterine insemination, IVF—in vitro fertilization, PGD—preimplantation genetic diagnosis

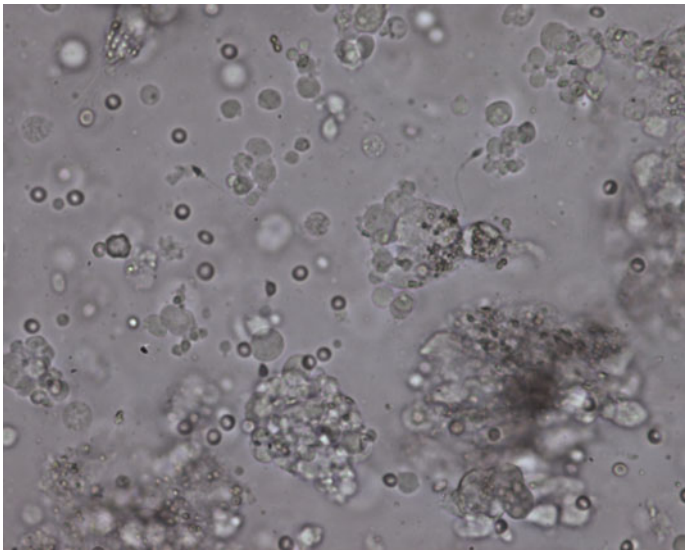

Fig. 16.7 Sperm aspirate: post-testicular sperm retrieval with motile sperms including supporting sertoli cells

functions as an ion channel and influences the formation of the ejaculatory duct, seminal vesicle, vas deferens, and distal two-thirds of the epididymis. The clinical diagnosis of absent vasa deferentia can be easily missed; therefore, men with azoospermia are carefully examined to exclude CBAVD—particularly those with a semen volume <1.5 mL and pH < 7.0 [26]. When a man has CBAVD, it is important to test both the partners for the cystic fibrosis (CF) gene for commonly observed mutations. If the female partner is found to be a carrier of CFTR mutations, the couple will be taken first for genetic counseling and very carefully proceeded with intracytoplasmic sperm injection (ICSI) using the male's sperm. The risk of having a child with CF or CBAVD will be 50%, depending on the type of mutations carried by the parents. If the female partner is negative for known mutations, the risk of being a carrier of unknown mutations will be $\sim$0.4%.

Assisted Reproductive Technologies

Intrauterine Insemination (IUI)

Intrauterine insemination (IUI) is one of the simple techniques of assisted reproductive technology (ART) for treating infertility by artificial insemination. Insemination can be performed using the male partner's sperm or with sperm from a donor, which is tested, frozen, quarantined, and then retested. This therapy is

relatively minimally invasive and an uncomplicated procedure. IUI involves the processing of semen in the laboratory so as to yield active sperm devoid of seminal plasma, which is then directly placed into the uterus. The separation of human spermatozoa from seminal plasma yields a final preparation containing a high percentage of morphologically normal and motile cells, free from debris, non-germ cells, and dead spermatozoa. Diluting semen with culture media and centrifuging is used for preparing normozoospermic specimens for IUI.

Sperm Preparation Technique

The choice of sperm preparation technique is dictated by the nature of the semen sample. The direct swim-up technique is often used when the semen samples are considered to be largely normal, whereas in the cases of severe oligozoospermia, tetatozoospermia, or asthenozoospermia, density gradients are usually preferred because of the greater total number of motile spermatozoa recovered.

Direct Swim-Up: Spermatozoa may be selected by their ability to swim out of seminal plasma and into culture medium. This is known as the "swim-up" technique. The direct swim-up of spermatozoa from semen is the preferred method of separating out the motile spermatozoa. Motile spermatozoa then swim into the culture medium. The procedure gives a lower yield of spermatozoa than washing, but selects them for their motility and is useful where the percentage of motile spermatozoa in semen is low, e.g., for IVF and ICSI.

Density Gradients: Discontinuous density gradients can be single gradient or double gradient that can provide the best selection of good quality spermatozoa, giving good separation from other cell types and debris. It is easier to standardize than the swim-up techniques, and thus, results are more consistent. These techniques are used to recover and prepare spermatozoa for use in IUI and IVF. This method uses centrifugation of seminal plasma over the single or double density gradients consisting of colloidal silica coated with silane, which separates cells by their density. In addition, motile spermatozoa swim actively through the gradient material to form a soft pellet at the bottom of the tube.

Sperm Aspiration Techniques

Sperm aspiration techniques involve the use of minor surgical procedures to collect sperm from organs within the genital tract. These techniques are indicated for men in whom the transport of sperm is not possible because the ductal system that normally carries sperm to the ejaculate is absent, such as with the congenital absence of the vas deferens. Presently, sources of sperm in otherwise azoospermic patients, or those with no ejaculated sperm, include the vas deferens, epididymis, and testicle, using sperm

aspiration techniques in which the sperm is suctioned from the organ. Some of the available sperm aspiration techniques are summarized as follows:

Testicular Sperm Extraction (TESE)

Testicular sperm extraction (TESE) is indicated for patients with blockage in the epididymis very near the testis. This condition can arise due to either prior surgery, infection, or from birth, or a blockage of efferent ductules. It is also indicated in men with extremely poor sperm production (rare sperms) and cannot reach the ejaculate (Fig. 16.8). In this procedure, a small amount of testis tissue is taken by biopsy under local anesthesia (Fig. 16.9). It is evident from this technique that sperm do not have to "mature" or pass through the epididymis in order to fertilize an egg, and therefore, testicular sperm utilizes ICSI technique for fertilization. Testicular biopsy or sperm extraction (TESE) is the part of ICSI treatment in patients with clinical evidence of NOA (Fig. 16.10). Hence TESE is the technique of choice for NOA patients. Spermatogenesis may be focal, which means that in about 50% of men with NOA, spermatozoa can be found and used for ICSI. Microsurgical TESE (micro-TESE) increases retrieval rates versus conventional TESE and should be preferred in severe cases of NOA [27–29]. One drawback of testis sperm is that it does not freeze as readily as epididymal or vasal sperm, so it is more likely that the male partner will need to undergo repeated procedures for each IVF attempt.

Vasal Aspiration

This sperm extraction technique is recommended in the patients with congenital or acquired obstruction of the ductal system at the level of the prostate or in the abdominal or pelvic portions of the vas deferens. Also the patients who have undergone a vasectomy less than 5 years or before can be included for vassal aspiration of sperms. It can be done through a small scrotal incision or through incisionless techniques. The vas deferens is entered, and a syringe is used to suction leaking sperm into a nourishing media, and more sperms are brought to the opening by gently massaging the epididymis and vas deferens. Aspirated sperm are specially processed and prepared for insemination for IVF. The vasal aspiration provides the most "mature" sperms as they have already passed through the epididymis, where maturation processes occur during normal sperm development (Figs. 16.11, 16.12, 16.13, 16.14, 16.15, and 16.16). The sperm aspirated from vasal are equivalent to ejaculated sperm and thus can be frozen at the time of surgery to avoid further procedures in the male.

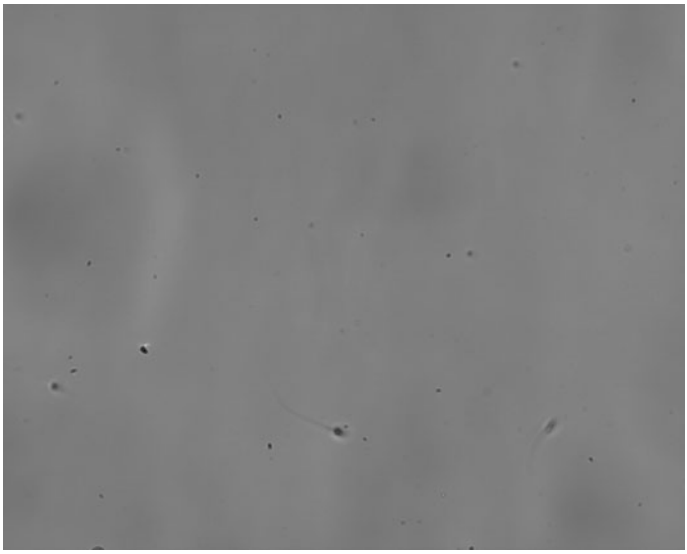

Fig. 16.8 Semen sample from severe oligozoospermic patients with occasional sperms or "rare sperms"

Fig. 16.9 Testicular aspiration sperm needle for TESE. (Cat. No: COOK K-TESE-19-3.0-NS-MARIBOR, Willian A COOK Australia PTY LTD, 95 Brandl Street. Eight Mile Plains, Brisbane, QLD 4113, Australia)

Fig. 16.10 SpermMobil used for surgical sperm retrieval preparation of vital immotile sperms for ICSI and diagnosis of necrozoospermia

Epididymal Aspiration

Epididymal sperm aspiration can be performed in situations in which the vas deferens is either absent or scarred from prior surgery, trauma, or infection. Sperm are directly collected from a single, isolated epididymal tubule (microsurgical epididymal sperm aspiration, MESA) or by blind needle puncture (percutaneous epididymal sperm aspiration, PESA) in much the same manner as the vasal procedure. Depending on the length of the epididymis that is available for aspiration, multiple separate aspiration attempts can be made from 1 or both testicles.

When 10 million–20 million sperm are obtained, the sperm are processed for fertilization of the partner's eggs. Epididymal sperm are not as "mature" as sperm and have traversed the entire length of the epididymis and reside in the vas deferens, and, as a consequence, epididymal sperm require ICSI to fertilize eggs. Like vasal sperm, these sperm can be frozen at the time of surgery to eliminate future surgical sperm retrieval procedures.

Microsurgical Epididymal Sperm Aspiration

The MESA technique is utilized to retrieve sperm from the proximal region of the epididymis (where the most active spermatozoa are located). This technique along with IVF was developed to treat patients with CBAVD and irreparable obstructive azoospermia as a major cause of male infertility. TESE and PESA are also viable options, but 1 MESA procedure provides sufficient material for several ICSI cycles,

and it produces high pregnancy and fertilization rates. Before microsurgery, epididymal spermatozoa should be aspirated and cryopreserved for use in ICSI [30, 31].

Fig. 16.11 HBA® slide provides an answer to the proportion of mature binding spermatozoa. (Cat. No BCT-HBA-10 HBA® slide, Manufactured by Biocoat, Inc, Distributed by ORIGIO a/s Knardrupvej 2, 2760 Måløv, Denmark)

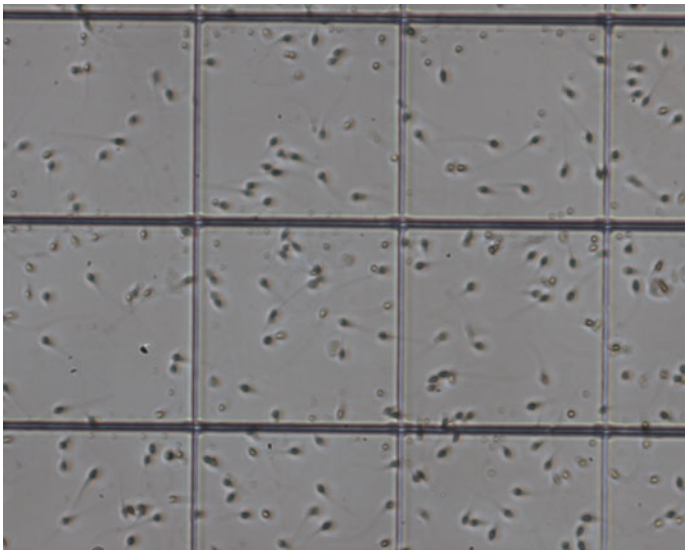

Fig. 16.12 Mature bound sperms on HBA® slides with normal morphology, integrity, and aneuploidy with rigorous tail movement with no forward motion of head

Fig. 16.13 PICSI® dish that binds mature sperm with high hyaluranon binding ability (Cat. No BCT-PICSI-20 20 PICSI® Dish, Manufactured by Biocoat, Inc, Distributed by ORIGIO a/s Knardrupvej 2, 2760Måløv, Denmark)

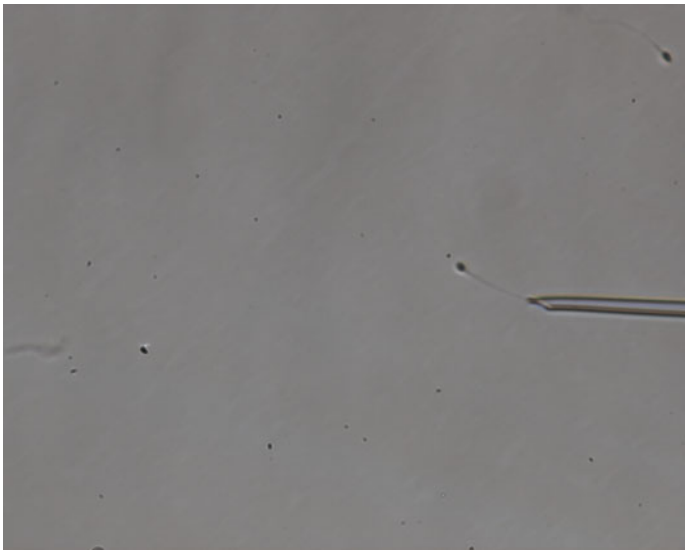

Fig. 16.14 PICSI procedure entails picking up of mature bound sperm from PICSI® dish

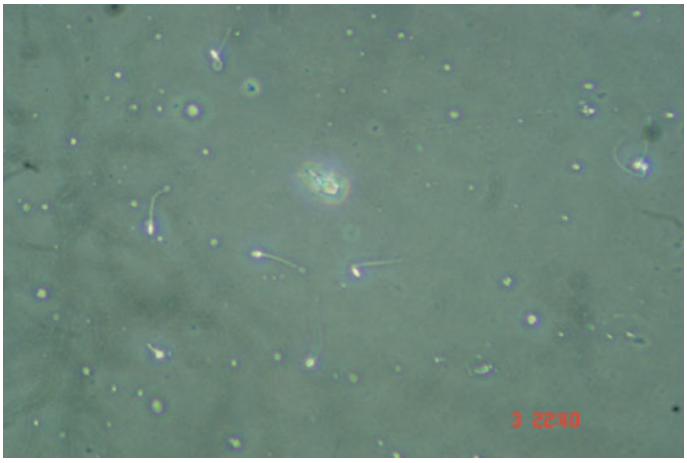

Fig. 16.15 Healthy spermatozoon showing swelling and curling of the tail region

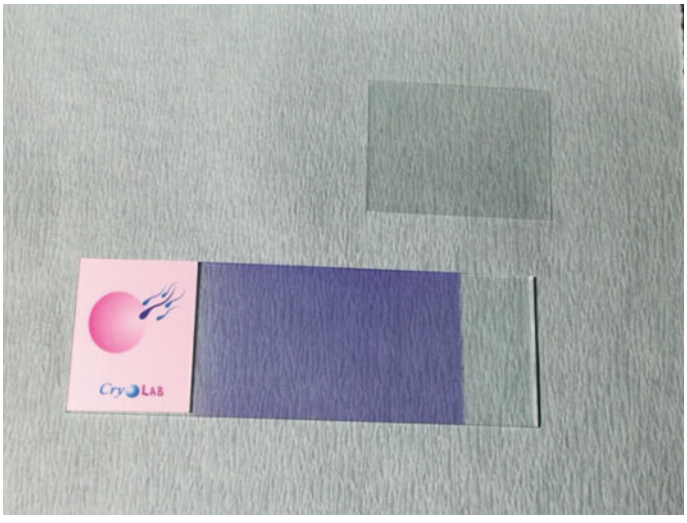

Fig. 16.16 Commercially available slides for assessing sperm morphology (Product information: easy Morpho kit; Manufacture by Cryo Lab International, Distributed by SAR Healthline Pvt Ltd., SAR House, Parayanchery, Calicut, India)

Percutaneous Epididymal Sperm Aspiration

PESA is another simple technique to obtain sperm for ICSI in men who have an obstruction of the vas deferens, due to either vasectomy or other obstruction (Figs. 16.17 and 16.18). To minimize scarring and damage, PESA usually is attempted on 1 side only. It is sometimes necessary to aspirate from both sides, and

sufficient sperm for ICSI is obtained in 80% of attempts. In 10% of cases, enough suitable sperm is found for cryopreservation. To locate the vas deferens, gently feel the scrotum; a small needle is inserted into the vas deferens and an assistant draws back the plunger to aspirate seminal fluid. When fluid is obtained, it is passed to the andrologist to examine the motile sperm.

In Vitro Fertilization (IVF)

The procedure requires that the female partner undergoes ovarian stimulation with fertility medications so that several mature eggs develop. These eggs are then suctioned through the vagina, using vaginal ultrasound, and incubated under precise conditions in the embryology laboratory. The semen sample is prepared by spinning the sperm cells through a special medium. This solution separates live sperm from debris and most of the dead sperm. Standard IVF requires the availability of adequate number of ejaculated spermatozoa to enable sperm preparation and inseminate each egg with 50×10^3.

Intracytoplasmic Sperm Injection (ICSI)

Intracytoplasmic sperm injection (ICSI) is a technique that is developed to help achieve fertilization for couples with severe male factor infertility or couples who have had previous fertilization failure. The procedure overcomes many fertilization barriers and allows couples with hope of achieving successful pregnancy by obtaining the fertilized embryos. The specialist picks up the single live sperm in a glass needle and injects it directly into the egg.

ICSI is a first choice in the couples who had no or poor fertilization after 2 IVF cycles. The studies have shown that aspirates with very good sperm concentration and motility are obtained from epididymis have low fertilization and pregnancy rate using routine IVF techniques [32]. It is further indicated in the non-ejaculated samples, i.e., spermatozoa aspirated from vas deferens, epididymis, or testicular samples where immotile spermatozoa are found. The cases with acrosome deficient (round-headed) spermatozoa, with immotile cilia syndrome with 100% immotile spermatozoa, are also indicated for doing ICSI. In these cases, eosin-nigrosin staining is done for vitality staining followed by HOS test to select viable spermatozoa for ICSI (Fig. 16.19).

The studies have demonstrated exceptionally high fertilization and pregnancy rates from cryopreserved spermatozoa obtained from epididymis and testis. These spermatozoa usually survive well in the freezer and retain good fertilization potential after thawing and by using ICSI as preferred technique for insemination [33]. Fertilization failure in the first IVF cycle or unexpected poor fertilization rate

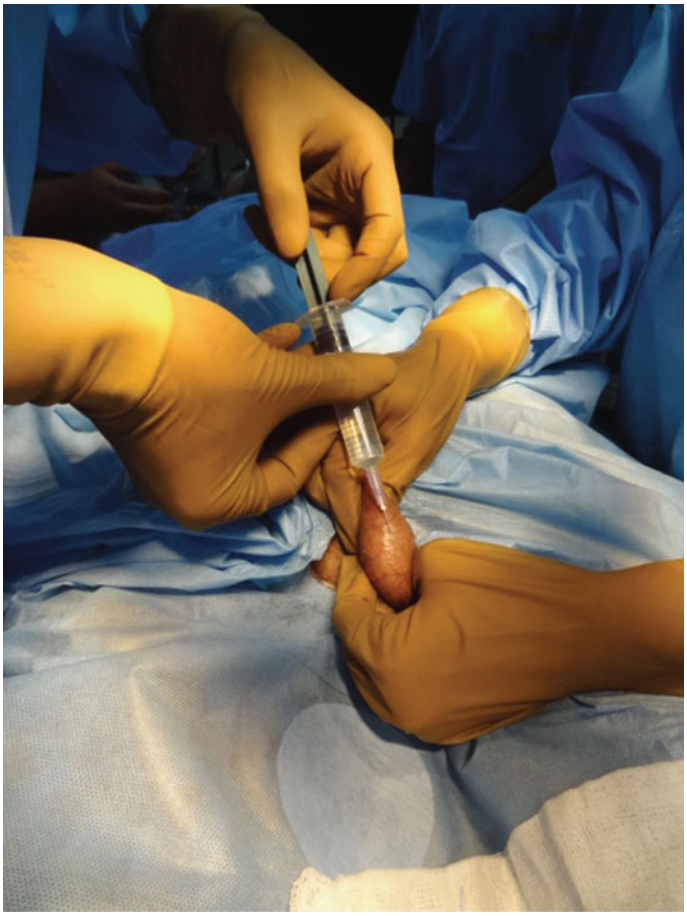

Fig. 16.17 Clinical procedure for sperm retrieval through percutaneous epididymal sperm aspiration (PESA)

(<15%) could be due to either failed oocyte activation or incomplete decondensation of spermatozoa [34]. It has been demonstrated during the studies that approach of splitting oocytes if the number exceeds 10 for IVF and ICSI in the same cycle may improve the fertilization rate and clinical pregnancy rate [35].

Semen Cryopreservation

Cryopreservation is the storage of biological material at subzero temperatures at −80 or −196 °C (the boiling point of liquid nitrogen) at which biochemical processes of cell metabolism are slowed or interrupted. At −196 °C, the biochemical

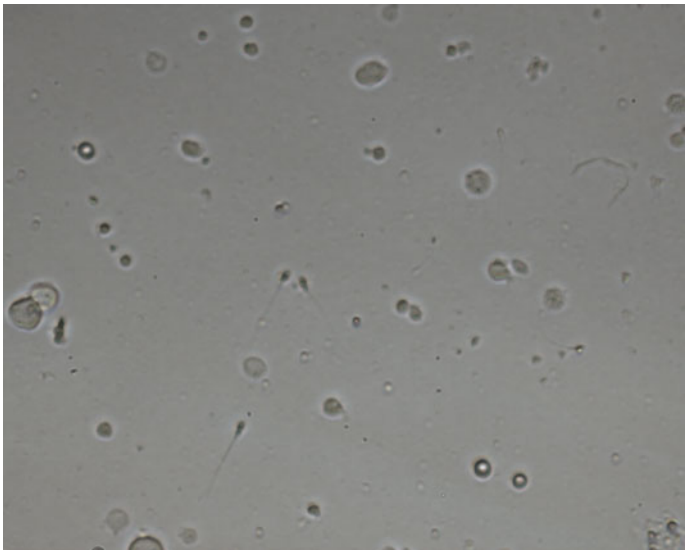

Fig. 16.18 Sperms aspirated through percutaneous epididymal sperm aspiration (PESA) technique from obstructive azoospermia patients

reactions that lead to cell death are stopped. Storage of sperm is indicated for the patient before potentially sterilizing chemotherapy or radiotherapy for cancer or for non-malignant diseases [36], and before surgery that might interfere with fertility (e.g., bladder neck surgery in a younger man or removal of a testicle in a man with testicular malignancy, or before vasectomy or transgender surgery). After gonadotropin treatment has induced spermatogenesis in men with hypogonadotrophic hypogonadism in men with NOA, the chance of finding sperm using micro-TESE is ~50%. Cryopreservation can be used for sperm collected through TESE and avoid repeated sperm retrieval procedures and unnecessary hyperstimulation of the female partner. Also, cryopreservation of donor semen samples reduces the risk of transmission of infection from sperm donors.

Preimplantation Genetic Screening (PGS)

Aneuploidy is the most common type of chromosome abnormality and a leading cause of implantation failure, miscarriage, and congenital abnormalities [37].

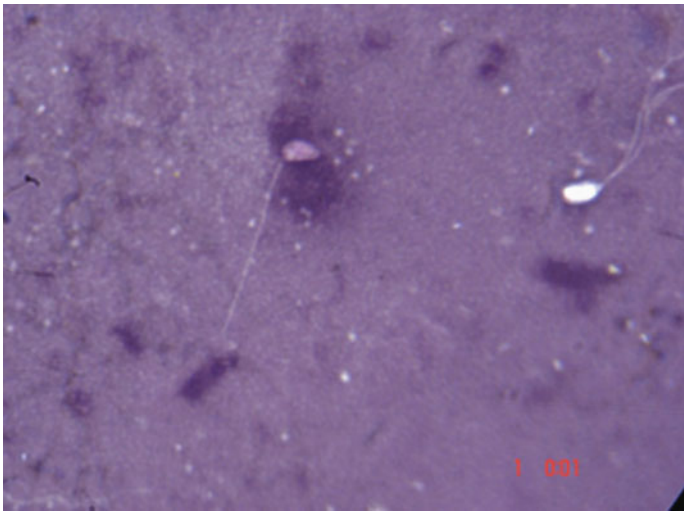

Fig. 16.19 Eosin-nigrosin staining: *Pink stained* sperms are non-viable sperms. Nigrosin provides *dark background*

Chromosome aneuploidy is the major cause of IVF failure as most embryos with aneuploidy will be of low grade and have implantation failure. There are also different embryological challenges responsible for fertilization failure and poor quality of embryos (Table 16.6). Preimplantation genetic screening (PGS) involves removal of 1 or 2 blastomeres from 6 to 8 cell embryos, or removal of trophectoderm cell at the blastocyst stage, followed by comprehensive aneuploidy analysis using array comparative genomic hybridization (CGH) to identify euploid embryos [38].

Artificial Insemination with Donor Semen (AID)

Donor insemination is 1 option for treating infertility due to male factor. It involves placing of a donor's semen, obtained from an accredited ART bank, inside the vagina or the cervix. The donated sperm is used to fertilize an egg either inside the woman's body (as in the case of intrauterine insemination) or in the laboratory in the case of IVF.

Table 16.6 Common embryological challenges while treating patients of male infertility

Embryological challenges	Laboratory observations	Additional diagnostic workup	Assisted reproductive technology —to choose	Clinical intervention
Fertilization failure	Sperms fails to fertilize mature oocyte thus leading to failed IVF outcome	Sperm–hyaluronan-binding assay provides a qualitative assessment of sperm quality, maturity, and fertilizing potential	Normal HBA scores (>60%) **Physiologic ICSI (PICSI)**	If HBA binding levels are low we shift to PICSI The most efficient sperm is attached to hyaluronan-coated microwells and is selected for **PICSI**
		Sperm DNA damage may result in lower fertilization rates, impaired implantation and increase in pregnancy loss rates and measure by sperm DNA integrity by semi-quantitative estimation of DNA Fragmentation Index (DFI) test through sperm chromatin dispersion (SCD)/halo test	**Low DFI (19%)-IUI/IVF** (*Sperms with low DFI have reduced chromosomal aneuploidies, DNA damage and miscarriage*) **High DFI ($\geq$ 20%) → ICSI**	Treat with antioxidant (AOX) and culture sensitive antibiotics Repeat DFI after 0–6 months Male with low DFI is taken for intrauterine insemination IUI/IVF Male with high DFI is enrolled for ICSI after 3–6 months treatment with AOX and antibiotics
Poor embryo quality/recurrent implantation failure	Poor embryo quality leading to implantation failure or cycle cancellation	Sperm aneuploidy analysis for chromosome aneuploidy DNA Fragmentation Index (DFI) test through sperm chromatin dispersion (SCD) or any recommended technique Reactive oxygen species (ROS) estimation	**Sperm Aneuploidy Analysis**-Aneuploidy in sperm, particularly sex chromosome aneuploidy, is associated with severe damage to spermatogenesis **Preimplantation Genetic Screening (PGS)** through fluorescence in situ Hybridization (FISH)/array comparative genomic hybridization (CGH)/ next genome sequencing (NGS) ROS estimation guides us about the quantitative or qualitative	If positive ROS test, High DFI, bacterial infection Start on **Anti-oxidant therapy**, and **Antibiotic treatment** × 3–6 months ↓ Repeat semen analysis after 3 month ↓ IVF/ICSI
				Embryo biopsy → blastomeres → genetic

(continued)

Table 16.6 (continued)

Embryological challenges	Laboratory observations	Additional diagnostic workup	Assisted reproductive technology —to choose	Clinical intervention
			inflammatory reaction in the semen sample	analysis (array CGH → biopsied euploid embryos are cryopreserved Euploid embryos → embryo transfer (ET) during next IVF cycle
Infected culture plates	Culture media dishes look turbid/hazy/infected post-insemination or ICSI	Semen sample is investigated for contaminated microorganism through microbial culture and peroxidase staining of the sample Infected culture media is sent for microbiological studies	Use of gentamycin supplemented media **Sperm Sample Preparation:** IVF/ICSI by discontinuous gradient method that removes most of the microorganisms in the final sperm sample Selection of a single sperm-ICSI may reduce the risk of contamination	Male administered receives antibiotic prophylaxis post-microbial culture reports are obtained ICSI may be of benefit

IVF—in vitro fertilization, HBA—hyaluronan-binding assay, ICSI—intracytoplasmic sperm injection, PICSI—physiologic intracytoplasmic sperm injection, IUI—intrauterine insemination, ROS—reactive oxygen species

Donated sperms can be used in many types of infertility treatment. The common indication for donor insemination due to male factor includes the following:

1. Non-obstructive azoospermia
2. The man has a history of hereditary genetic disorder
3. The couple has Rh incompatibility
4. The man has a very poor semen parameter with azoospermia, oligospermia, or poor motility, and the couple does not wish to undergo any of the sophisticated ART such as ICSI

Conclusion

Over a period of time, due to advancements in ART, it has been observed that the sperm requirement for egg fertilization has dropped from hundreds of thousands for IVF to 1 viable sperm required through ICSI technique. This has also led to the recent development in ART for aggressive new surgical techniques to provide viable sperm for egg fertilization from men with low or no sperm count.

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Note: Page numbers followed by *f* and *t* indicate figures and tables, respectively.

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