Peter N. Schlegel · Bart C. Fauser
Douglas T. Carrell · Catherine Racowsky
Editors

Biennial Review of Infertility

Volume 3
2013

Springer

Biennial Review of Infertility

Peter N. Schlegel • Bart C. Fauser
Douglas T. Carrell • Catherine Racowsky
Editors

Biennial Review of Infertility

Volume 3

Springer

Editors
Peter N. Schlegel
Department of Urology
Weill Cornell Medical Center
New York Presbyterian Hospital
New York, NY, USA

Douglas T. Carrell
University of Utah School of Medicine
Salt Lake City, UT, USA

Bart C. Fauser
Department of Reproductive Medicine
University Medical Center Utrecht
Utrecht, The Netherlands

Catherine Racowsky
Department of Obstetrics and Gynecology
Division of Reproductive Endocrinlogy
and Infertility
Brigham and Women's Hospital
Boston, MA, USA

ISBN 978-1-4614-7186-8 ISBN 978-1-4614-7187-5 (eBook)
DOI 10.1007/978-1-4614-7187-5
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013938378

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Volume 3 of Biennial Review of Infertility is dedicated to the spirit of lifelong service to patients, trainees, and colleagues exemplified by Dr. Arnold Belker.

Preface

This third edition of Biennial Reviews of Fertility continues to build on a reputation of overviewing evolving fields that are important for the field of Reproductive Medicine. Each chapter is written by a leader in the field, who provides critical analysis of the developing subject for readers interested in staying on top of each area. Although books are typically viewed as having a longer "publication lag," limiting how timely the subject matter can be, the compilation of expert-reviewed cutting edge topics in this book is unique. For this reason, our reviews are updated biennially.

Since the "jury is still out" on a number of cutting edge topics, we have expanded our section of "Controversies." This portion of the book aims to provide critical insights on newer areas of investigation or treatment by having two different experts provide point–counterpoint evaluation of important topical subjects. In this issue, we are fortunate to have a balanced discussion of the issue of the safety of the ICSI procedure by its inventor, Dr. Gianpiero Palermo, with balanced inputs from both Doug Carrell and Kurt Barnhart. The role of IUI in modern reproductive medicine is debated by senior authors Erica Johnstone and Fulco van der Veen. Dr. Juergen Liebermann addresses the role of vitrification of human oocytes. The provocative topic of another chapter is, "Should we eliminate fresh embryo transfer from ART," addressed by Catherine Racowsky, Dan Kaser, and Maria Assens.

The role of aging in reproduction is addressed for both male and females by Kenneth Aston and Stephanie Sherman, respectively. Other topics include the role of sperm retrieval for couples with prior failed ART attempts, thoughtfully reviewed by Robert Oates, with an overview of the most recent meta-analyses of supplements for male infertility by Peter Schlegel. Dr. Raphi Ron-El covers the ethical issues and extent of Reproductive Tourism, a growing topic of special significance in European countries where substantial restrictions on reproductive options have been introduced.

Not only do our chapters cover every area from female reproduction to genetics to male reproduction to assisted reproduction, but we have also added a section on study design to help our readers better interpret published literature in reproductive medicine. In this volume, the role of prospective cohort study design for trials in reproductive health is discussed by Stacey Missmer and Germaine Buck-Louis.

Each topic is obviously presented by a leader in the field of reproductive medicine. We thank our authors for the very short time line that is required for production of a timely set of reviews and the obvious other commitments that these authors have in our field. We appreciate the thoughtful and critical insights provided by our authors and hope that you recognize the value of these efforts as well.

New York, NY, USA — Peter N. Schlegel, M.D.
Utrecht, The Netherlands — Bart C. Fauser, M.D.
Salt Lake City, UT, USA — Douglas T. Carrell, Ph.D.
Boston, MA, USA — Catherine Racowsky, Ph.D.

Contents

Contributors

Emily G. Allen, Ph.D. Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA

Maria Assens, M.D. Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Kenneth I. Aston, Ph.D., H.C.L.D. Andrology and IVF Laboratories, Division of Urology, Department of Surgery, University of Utah School of Medicine, Salt Lake City, UT, USA

Valerie L. Baker, M.D. Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Stanford University School of Medicine, Palo Alto, CA, USA

Kurt T. Barnhart, M.D., M.S.C.E. Penn Fertility Care, Department of Obstetrics and Gynecology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Women's Health Clinical Research Center, Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA

Lora J.H. Bean, Ph.D. Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA

Frank J.M. Broekmans, M.D. Department of Reproductive Medicine and Gynaecology, University Medical Center Utrecht, CX Utrecht, The Netherlands

Douglas T. Carrell, Ph.D., H.C.L.D. Andrology and IVF Laboratories, Department of Surgery (Urology), University of Utah School of Medicine, Salt Lake City, UT, USA

Department of Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, UT, USA

Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT, USA

Peter T.K. Chan, M.D., C.M., M.Sc., F.R.C.S.(C), F.A.C.S. Male Reproductive Medicine, Department of Urology, McGill University Health Center, Montreal, QC, Canada

Ching-Chien Chang, Ph.D. Reproductive Science Center, University of Massachusetts, Lexington, MA, USA

Jessie Dorais, M.D. Reproductive Endocrinology and Infertility, Utah Center for Reproductive Medicine, University of Utah, Salt Lake City, UT, USA

Lisa Dovere, B.S. GENERA Centre for Reproductive Medicine, Clinica Valle Giulia, Rome, Italy

Bart C. Fauser, M.D., Ph.D. Department of Reproductive Medicine and Gynaecology, University Medical Center Utrecht, Utrecht, The Netherlands

Trina Fields, B.S. The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY, USA

Antonio R. Gargiulo, M.D. Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School, Boston, MA, USA

Center for Robotic Surgery, Brigham and Women's Health Care, Center for Infertility and Reproductive Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Kathryn J. Go, Ph.D. Reproductive Science Center, University of Massachusetts, Lexington, MA, USA

Department of Obstetrics and Gynecology, University of Massachusetts Medical School, Worcester, MA, USA

Clarisa R. Gracia, M.D., M.S.C.E. Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA

Timothy G. Jenkins, B.S. Andrology and IVF Laboratories, Department of Surgery, University of Utah School of Medicine, Salt Lake City, UT, USA

Erica B. Johnstone, M.D., M.H.S. Reproductive Endocrinology and Infertility, Utah Center for Reproductive Medicine, University of Utah, Salt Lake City, UT, USA

Suleena Kansal Kalra, M.D., M.S.C.E. Penn Fertility Care, Department of Obstetrics and Gynecology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Daniel J. Kaser, M.D. Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Benjamin Leader, M.D., Ph.D. Clinical Research Division, ReprosSource Inc., Woburn, MA, USA

Juergen Liebermann, Ph.D., H.C.L.D. In Vitro Fertilization Laboratory, Fertility Centers of Illinois, River North Center, Suite, Chicago, IL, USA

Shane T. Lipskind, M.D. Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School, Boston, MA, USA

Germaine M. Buck Louis, Ph.D., M.S. Division of Epidemiology, Statistics and Prevention Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Rockville, MD, USA

Roberta Maggiulli, B.S. GENERA Centre for Reproductive Medicine, Clinica Valle Giulia, Rome, Italy

Stacey A. Missmer, Sc.D. Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women's Hospital, Harvard Schools of Medicine and Public Health, Boston, MA, USA

Lobke M. Moolenaar, M.D. Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Zsolt Peter Nagy, Ph.D. Reproductive Science Center, University of Massachusetts, Lexington, GA, USA

Queenie V. Neri, M.Sc. The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY, USA

Robert D. Oates, M.D. Department of Urology, Boston University School of Medicine and Boston Medical Center, Boston, MA, USA

Gianpiero D. Palermo, Ph.D., M.D. The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY, USA

Catherine Racowsky, M.D. Department of Obstetrics and Gynecology, Division of Reproductive Endocrinlogy and Infertility, Brigham and Women's Hospital, Boston, MA, USA

Laura Rienzi, M.Sc. GENERA Centre for Reproductive Medicine, Clinica Valle Giulia, Rome, Italy

Raphael Ron-El, M.D. Fertility and IVF Unit, Department of Obstetrics & Gynecology, Assaf Harofeh Medical Center, Sackler Medical School, Tel Aviv University, Tel Aviv, Israel

Zev Rosenwaks, M.D. The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY, USA

Peter N. Schlegel, M.D. Department of Urology, Weill Cornell Medical Center, New York Presbyterian Hospital, New York, NY, USA

Suneeta Senapati, M.D. Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA

Stephanie L. Sherman, Ph.D. Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA

Helen L. Torrance, M.S. Department of Reproductive Medicine and Gynaecology, University Medical Center Utrecht, CX Utrecht, The Netherlands

Filippo Ubaldi, M.D., M.Sc. GENERA Centre for Reproductive Medicine, Clinica Valle Giulia, Rome, Italy

Theodora C. van Tilborg, M.D. Department of Reproductive Medicine and Gynaecology, University Medical Center Utrecht, CX Utrecht, The Netherlands

Bradley J. Van Voorhis, M.D. Center for Advanced Reproductive Care, University of Iowa, Iowa City, IA, USA

Fulco van der Veen, M.D., Ph.D. Amsterdam Academic Medical Centre, University of Amsterdam, Amsterdam, North-Holland, The Netherlands

Rachel Weinerman, M.D. Penn Fertility Care, Department of Obstetrics and Gynecology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

List of Abbreviations

3D	Three-dimensional
AAGL	American Association for Gynecologic Laparascopists
AC	Artificial collapsing
AFC	Antral follicle count
AM	Abdominal myomectomy
AMH	Anti-Mullerian hormone
ART	Assisted reproduction technologies
AS	Angelman Syndrome
ASD	Autism spectrum disorders
ASDP	Atlanta Down Syndrome Project
ASRM	American Society for Reproductive Medicine
BMP-15	Bone morphogenetic protein-15
BWS	Beckwith–Wiedemann Syndrome
CAG	Cystone adenine guanine
CBRC	Cross-border reproductive care
CC	Clomiphene citrate
CCCT	Cytosine (×3) thymine
CCSS	Childhood cancer survivorship study
CDC	Centers for Disease Control and Prevention
CET	Cryopreserved embryo transfer
CI	Confidence interval
CLIA	Clinic Laboratory Improvement Act
COH	Controlled ovarian hyperstimulation
CONSORT	CONSistentcy in r-FSH starting dOses for individualized tReatmenT
COS	Controlled ovarian stimulation
CP	Cerebral palsy
CPA	Crioprotectant
CPAP	Continuous positive airway pressure
cPR	Clinical pregnancy rate
DES	Diethylstilbestrol
DFI	DNA Fragmentation Index
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DM	Myotonic dystrophy

DMSO	Dimethyl sulphoxide
DNA	Decoy ribonucleric acid
DNAH11	Dynein heavy chain 11
DNAH5	Dynein heavy chain 5
dUTP	Deoxynucleotidyl transferase-mediated
E2	Estradiol
EFORT	Exogenous FSH ovarian reserve test
EG	Ethylene glycol
eNOS	Endothelial nitric oxide synthase
ERCP	Endoscopic retrograde cholangiopancreatography
ES	Equilibration solution
eSET	Elective single embryo transfer
ET	Embryo transfer
EUROCAT	European surveillance of congenital anomalies
FASTT	Fast track and standard treatment trial
FDA	United States Food and Drug Administration
FET	Frozen embryo transfers
FORT-T	Forty and over infertility treatment trial
FSH	Follicle-stimulating hormone
GEE	Generalized estimating equations
GGC	Guanine guanine cytosene
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
GO	Glass oviduct
GUTS	Growing Up Today Study
hCG	Human chorionic gonadotropin
HDL	High densitylipoprotein
hMG	Human menopausal gonadotropins
HOMA	Homeostatic model assessment
HPO	Hypothalamic pituitary ovarian
HSV	High security vitrification kit
HSV	Hemi-straw system
HTF	Human tubal fluid
HTT	Huntingtin gene
ICMART	International Committee for Monitoring Assisted Reproductive Technology
ICSI	Intracytoplasmic sperm injection
IHH	Idiopathic hypogonadotropic bypogonadism
IMSI	Intracytoplasmic morphologically selected sperm injection
IPSS	International prostate symptom score
IQ	Intelligence quotient
IR	Implantation rate
IUI	Intra uterine insemination
IVF	In-vitro fertilization
LDH	Lactate dehydrogenase
LH	Luteinizing hormone
LM	Laparoscopic myomectomy
LOH	Late-onset hypogonadism

LUTS	Lower urinary tract symptoms
MDI	Mental development index
MEN	Multiple endocrine neoplasia
MENT	Methyl nortestosterone
MESA	Microsurgical epididymal sperm aspiration
MI	Meiosis I
MII	Meiosis II
MRI	Magnetic resonance imaging
MTHF	Methylene tetrahydrofolate
NADP	Nicotinamide adenine dinucleotide
NASA-TLX	National Aeronautics and Space Administration Task Load Index
NC	Non cohort
NDSP	National Down Syndrome Project
OAT	Oligo-astheno-teratospermia
ODS	Ovarian dysgenesis syndrome
OHSS	Ovarian hyper-stimulation syndrome
oPR	Ongoing pregnancy rate
OPS	Open pulled straw
OPTIMIST	OPTIMisation of cost effectiveness through Individualized FSH STimulation
OR	Odds ratio
ORT	Ovarian reserve test
PADAM	Partial androgen deficiency in aging men
PCOS	Polycystic ovarian syndrome
PDE5I	Phostphodiesterase-5 inhibitors
PDMS	Polydimethylsiloxane
PEMT	Phosphatidylethanolamine *n*-methyl transferase
PESA	Percutaneous epididymal sperm aspiration
POI	Primary ovarian insufficiency
POR	Poor ovarian response
PPV	Positive predictive value
PSA	Prostate specific antigen
PZD	Partial zona dissection
RCT	Randomized controlled trial
REI	Reproductive endocrinology and infertility
RFID	Radio frequency identification
rFSH	Recombinant follicle stimulating hormone
rLH	Recombinant luteinizing hormone
RM	Robot-assisted laparoscopic myomectomy
ROS	Reactive oxygen species
RR	Relative risk
RTR	Robotic tubal reanastomosis
SA/V	Surface area-to-volume
SART	Society of Assisted Reproductive Techniques
SCD	Sperm chromatin dispersion test
SCSA	Sperm chromatin structure assay
SES	Social economic status

SHBG	Sex hormone binding globulin
SITA	Standard infertility treatment algorithm
SRS	Society of Reproductive Surgeons
SSS	Synthetic serum substitute
STS	Small tandem repeat
SUZI	Sub-zonal sperm injection
TB	Testosterone buciclate
TDS	Testicular dysgenesis syndrome
TECS	Tilting embryo culture system
TESA	Testicular sperm aspiration
TESE	Testicular sperm extraction
TIC	Timed intercourse
TRT	Testosterone replacement therapy
TTP	Time-to-pregnancy
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
uFSH	Purified urinary follicle stimulating hormone
USPHS	US Public Health Service
VS	Vitrification solution
WHO	World Health Organization
WOW	Well of the Well

Part I

Male Infertility

Supplements to Enhance Male Fertility

1

Peter N. Schlegel

1.1 Introduction

Nutritional supplements are not regulated by the Food and Drug Administration and are distributed from a wide variety of different manufacturers. Because sperm are known to be highly susceptible to oxidation, it is possible that antioxidant materials could protect sperm, limit sperm DNA damage, or enhance sperm function, including motility [1, 2]. Unfortunately, limited studies have evaluated the role of nutritional supplements in male fertility. Because such limited studies have been published, it is possible, and quite likely, that a publication bias exists towards positive studies. A recent Cochran meta-analysis reported the benefit of nutritional supplements for male fertility based on only 20 live births [3]. In addition, most studies on male supplements involve combination agents, making the benefit of any individual agent difficult to determine. In this analysis, we will discuss some of the in vitro effects of nutritional agents on sperm, as well as clinical trials for male infertility patients who are attempting to conceive naturally, and emphasize clinical trials of treatment prior to assisted reproduction. The antioxidant agents that have been described for potential use will be reviewed as well.

P.N. Schlegel, M.D. (✉)
Department of Urology, Weill Cornell Medical Center, New York Presbyterian Hospital, 525 East 68th Street, Starr 900, New York, NY 10065, USA
e-mail: pnschleg@med.cornell.edu

1.2 Antioxidant Agents

The following agents have been described as being nutritional supplements and represent vitamins, minerals, and other substances that may have a role in protecting sperm, enhancing sperm function, or potentially improving fertility both naturally and/or after assisted reproduction [4]. Each of these agents will be reviewed in terms of its mode of action and studies involving these agents presented.

1.2.1 Vitamin C

Vitamin C is a high potency water-soluble reactive oxygen species scavenger. It has been shown to neutralize superoxide, hydroxyl, and hydrogen peroxide radicals. It is naturally concentrated in semen at levels that are tenfold higher than that seen in serum. Systemic therapy with vitamin C decreases sperm DNA fragmentation, as measured by the presence of DNA adducts in sperm. It may also influence the expression of genes involved in intracellular redox pathways [5]. Of note, vitamin C can act as a pro-oxidant at high doses.

1.2.2 Vitamin E

Vitamin E is known to be a lipid-soluble antioxidant that is present in cell membranes. The presence of vitamin E protects the integrity of the phospholipid bilayer of the cell membrane as

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_1, © Springer Science+Business Media New York 2013

well as the mitochondrial sheath. In part, it acts as an antioxidant by interrupting the chain reaction of lipid peroxidation. Vitamin E can increase production of scavenger antioxidant enzymes, and it enhances the antioxidant activity of other agents. In vitro, it is known to protect sperm during cryopreservation [6].

1.2.3 Zinc

Zinc is a necessary mineral for optimal functioning of antioxidant enzymes, including superoxide dismutase. It inhibits membrane oxidative enzymes, such as NADP oxidase. It may also have a role in supporting the immunological system. It is well documented that lower zinc levels are present in the semen of infertile males and zinc deficiency has been associated with abnormal flagellae and microtubular defects in sperm. It is not clear, since zinc levels are so high in semen to begin with, whether the relative zinc deficiency seen in infertile males is enough to affect the natural function of this mineral. Systemic therapy is associated with reduced seminal fluid oxidative activity, apoptotic markers, and DNA fragmentation with a trend towards semen parameters [7].

1.2.4 Selenium

Selenium is a mineral that is required for normal testicular development, spermatogenesis, sperm motility, and function [8]. It reduces antioxidative stress by an unknown mechanism. Enzymes require selenium for normal function, including those that are involved in antioxidative pathways, such as phospholipid, hydroperoxide, glutathione peroxidase. Selenium administration increases glutathione peroxidation-1 expression, which destroys hydrogen peroxide, a potent oxidative agent.

1.2.5 Folate

Folate reduces homocysteine concentrations by its free radical scavenging properties. It may work synergistically with zinc to improve semen quality. It is known that defects in folate synthesis, such as defects in MTHF reductase or PEMT enzymes, are associated with male infertility. There is limited evidence for a role of folate deficiency in idiopathic male infertility [9].

1.2.6 Carnitine

Carnitine is a water-soluble antioxidant that is also our primary fuel for sperm motility. Carnitine is involved in the transport of long chain fatty acids into the mitochondrial matrix, possibly explaining its role in supporting sperm motility. Carnitine increases expression of antioxidant enzymes, including heme oxygenase-l and endothelial nitric oxide synthase (eNOS). Carnitine enhances cellular energetics in mitochondria by facilitating the free fatty acid entry into that organelle. Carnitines are thought to protect sperm DNA and cell membranes from reactive oxygen species induced DNA damage and apoptosis [10].

1.2.7 Carotenoids

Carotenoids work synergistically with selenium and vitamin E as antioxidants. The most commonly studied carotenoid is lycopene that is naturally derived from fruits and vegetables and found in especially high concentration in tomatoes. Carotenoids have a high reactive oxygen species quenching rate and are found in higher plasma levels than beta-carotene. High lycopene concentrations are found in the testes and seminal plasma. An additional carotenoid has been described recently, astaxanthin, a carotenoid extracted from algae. This agent has a high number of conjugated double bonds, making it a potent antioxidant. It is a more potent antioxidant than vitamin E or carnitine. Its role in male fertility has only recently been explored [11].

1.2.8 Coenzyme Q10 (Ubiquinone)

Coenzyme Q10 functions in electron transport and is an antioxidant. It is thought to be important

in mitochondrial function. It is found at high levels in metabolically active tissues. The semen level of coenzyme Q10 correlates with sperm concentration and motility, suggesting an intrinsic role in the production of sperm and sperm motility. Treatment of patients with coenzyme Q10 was associated with improved sperm concentration (OR = 1.6–5.5) after 6–9 months of treatment. It is also associated with improved sperm motility (OR = 1.4–4.5). In a small trial, couples where the male was treated with coenzyme Q10 resulted in nine pregnancies versus no pregnancies in the control group (OR = 2.2, $p = 0.24$) [12, 13]. Coenzyme Q10 is suggested to have a benefit on sperm production.

1.3 Quality of Antioxidant Trials

Most antioxidant trials have not been performed in a rigorous, randomized, controlled fashion. The scientific quality of antioxidant trials to-date has been relatively poor, as summarized by Ross et al. [4]. In most studies, the randomization method was not clear and allocation concealment was not clear as well. Double blinding was done for most of the studies, and no intention to treat analysis was done in the majority of the studies. Follow-up was typically strong with most studies reporting 90–100 % follow-up rate. Interpretation of these studies was often difficult because multiple agents were used and in some cases no placebo was applied. For example, in one study by Omu et al. [7], vitamin C, vitamin E, zinc, and other combinations of agents were used together. Similarly, Scott et al. [14] used vitamin A, vitamin C, vitamin E, and selenium, many of which have not been demonstrated to have antioxidant activities. Although most studies have suggested an odds ratio for effect of agents that was >1, the exact benefit, if any, of antioxidant therapies is not clear, in large part because of likely potential publication bias. In other words, studies were most likely to have been published if they demonstrated a benefit of intervention.

1.4 Results of Trials

1.4.1 Menevit

One of the most interesting interventional studies was a randomized controlled trial of antioxidants prior to IVF in a series of patients where the man had abnormal sperm DNA fragmentation. A total of 60 couples were enrolled. The men were treated with lycopene 6 mg, vitamin E 400 IU, vitamin C 100 mg, zinc 25 mg, selenium 26 mcg, folate 0.5 mg, and garlic 1,000 mg in palm oil vehicle. The placebo arm received palm oil vehicle alone. There was a 2:1 randomization of drug versus placebo and treatment was provided for 3 months before IVF-ICSI. Couples had to have had a prior failed IVF attempt and abnormal semen parameters, suggesting oxidative stress with abnormal sperm DNA fragmentation. The mean pre-treatment DFI was 39 % and female age was less than 39. The primary outcome was reported to be embryo quality.

Unfortunately, no difference was seen in embryo quality, and the pregnancy rate was not statistically different (per embryo transfer). However, the "viable pregnancy rate" differed between treatment and placebo groups, defined as ongoing pregnancy per embryo transferred, 46 % versus 24 %. Interestingly, the raw implantation rate in the treatment and control groups was not different ($p = 0.06$), and the raw biochemical pregnancy rate was not different ($p = 0.08$). Although the treatment was presumed to affect sperm DNA fragmentation, there was actually no repeat evaluation of sperm DNA fragmentation during treatment, raising a question as to whether any benefits or treatment were modulated by a direct effect on sperm [15].

1.4.2 Vitamin E and Zinc

The Cochran collaboration reported an evaluation of antioxidants on ART outcome. Any dose or type of antioxidant could be compared to placebo

or no treatment. The primary outcomes were analyzed in only three studies for live births. A secondary outcome, pregnancy rate, was evaluable in 15 studies. The Cochran meta-analysis demonstrated an odds ratio (OR) of 4.85, benefiting the use of oral antioxidants (95 % confidence interval 1.9–12.2) for a beneficial effect on live birth rates. The pregnancy rate was improved by an OR of 4.8 (2.6–6.6) in favor of antioxidant use. Interestingly, each of the studies looking at live birth had a positive result with comparisons involving vitamin E versus placebo [16, 17] and oral zinc versus no treatment [7]. Overall, only 18 out of 116 experimental arm patients achieved a live birth with 2 out of 98 in the control arm. Despite analysis of 34 trials involving 2,876 couples undergoing ART, the primary outcome for this meta-analysis could be determined by only three trials. A total of 20 live births occurred in these three trials. Both zinc and vitamin E were used. Of note, there is significant concern about high dose of vitamin E use and its cardiovascular risk [18]. Interestingly, in two of the trials, there were no pregnancies in the control arm. It is quite unusual for an ART intervention trial to have no pregnancies in a control arm. Using pregnancy rate as an outcome, a larger number of studies were involved but the antioxidants used ranged from multiple agents, to vitamin E, to L-acetylcarnitine plus L-carnitine, L-carnitine alone, vitamin C and vitamin E, magnesium, coenzyme Q10, and zinc. In the meta-analysis for pregnancy rates, a total of 53 pregnancies were analyzed.

When the pregnancy rate was evaluated as an outcome (admittedly, a secondary outcome for this planned Cochran analysis) the magnitude of benefit appeared to be greater than the effect that would be expected by improving sperm DNA fragmentation. Antioxidants are thought to function by decreasing sperm DNA fragmentation, and the magnitude of benefit (OR for pregnancy with treatment) was 4.18. One meta-analysis of the effect of DNA fragmentation on pregnancy rates during ART reported a diagnostic OR of 1.44 [19]. Therefore, the magnitude of benefit (400 %) appeared to greatly outweigh the magnitude of benefit that would be suggested from DNA fragmentation alone (44 %). Of note, the pregnancy rate in the control group was 0–11 % in most of the trials with 3 % as a mean value (versus 16 % in the treatment group). This very low pregnancy rate after assisted reproduction suggests some concern with the type of ART performed or the site for these trials.

Taken together, only 20 pregnancies were involved in demonstrating the treatment effect that is proposed in the Cochran meta-analysis, from a total of three trials. The risk of publication bias appears to substantially affect the purported benefit of this intervention. Multiple agents were considered together to evaluate this effect. Even though the magnitude of benefit (OR = 4.8) for live births suggests benefit, it is not clear how to interpret these results.

1.5 Summary

Antioxidants appear to have some promise as agents that could provide a benefit of improving fertility potential for men with abnormal sperm DNA fragmentation, and possibly men with idiopathic infertility. The most promising agents appear to be vitamin E, carnitines, astaxanthin, vitamin C, zinc, and possibly coenzyme Q10. Unfortunately, based on published data, it is impossible to make evidence-based recommendations of a specific agent, dose, or concoction of supplements for a couple with male factor infertility. What dose should be used, what combination of agents, and the actual mechanism of action is impossible to determine from published data. All that one can say at this point is that antioxidants might have benefit in the treatment of male infertility, especially for men with abnormal sperm DNA fragmentation or idiopathic infertility. Unfortunately, the magnitude of benefit and treatment regimen to be recommended is yet to be determined.

References

1. Lewis SE. Is sperm evaluation useful in predicting human fertility? Reproduction. 2007;134(1):31–40.
2. Kefer JC, Agarwal A, Sabanegh E. Role of antioxidants in the treatment of male infertility. Int J Urol. 2009;16(5):449–57.

3. Showell MG, Brown J, Yazdani A, et al. Antioxidants for male subfertility. Cochrane Database Syst Rev. 2011;1:CD007411.
4. Ross C, Morriss A, Khairy M, et al. A systematic review of the effect of oral antioxidants on male infertility. Reprod Biomed Online. 2010;20(6):711–23.
5. Fraga CG, Motchnik PA, Shigenaga MK, et al. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. Proc Natl Acad Sci USA. 1991;88(24):11003–6.
6. Dawson EB, Harris WA, Rankin WE, et al. Effect of ascorbic acid on male fertility. Ann N Y Acad Sci. 1987;498:312–23.
7. Omu AE, Al-Azemi MK, Kehinde EO, et al. Indications of the mechanisms involved in improved sperm parameters by zinc therapy. Med Princ Pract. 2008;17(2):108–16.
8. Ursini F, Heim S, Kiess M, et al. Dual function of the selenoprotein PHGPx during sperm maturation. Science. 1999;285(5432):1393–6.
9. Murphy LE, Mills JL, Molloy AM, et al. Folate and vitamin B12 in idiopathic male infertility. Asian J Androl. 2011;13(6):856–61.
10. Cavallini G, Ferraretti AP, Gianaroli L, et al. Cinnoxicam and L-carnitine/acetyl-L-carnitine treatment for idiopathic and varicocele-associated oligoasthenospermia. J Androl. 2004;25(5):761–70. discussion 71–2.
11. Klebanov GI, Kapitanov AB, Teselkin Yu O, et al. The antioxidant properties of lycopene. Membr Cell Biol. 1998;12(2):287–300.
12. Balercia G, Regoli F, Armeni T, et al. Placebo-controlled double-blind randomized trial on the use of L-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine in men with idiopathic asthenozoospermia. Fertil Steril. 2005;84(3):662–71.
13. Safarinejad MR, Safarinejad S. Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: a double-blind, placebo controlled, randomized study. J Urol. 2009;181(2):741–51.
14. Scott R, MacPherson A, Yates RW, et al. The effect of oral selenium supplementation on human sperm motility. Br J Urol. 1998;82(1):76–80.
15. Tremellen K, Miari G, Froiland D, et al. A randomised control trial examining the effect of an antioxidant (Menevit) on pregnancy outcome during IVF-ICSI treatment. Aust N Z J Obstet Gynaecol. 2007;47(3):216–21.
16. Kessopoulou E, Powers HJ, Sharma KK, et al. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated male infertility. Fertil Steril. 1995;64(4):825–31.
17. Suleiman SA, Ali ME, Zaki ZM, et al. Lipid peroxidation and human sperm motility: protective role of vitamin E. J Androl. 1996;17(5):530–7.
18. Bjelakovic G, Nikolova D, Gluud LL, et al. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA. 2007;297(8):842–57.
19. Collins JA, Barnhart KT, Schlegel PN. Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? Fertil Steril. 2008;89(4):823–31.

Poor Quality Ejaculate Sperm: Do the Data Support the Use of Testis Sperm?

2

Robert D. Oates

2.1 Introduction

Many cases of male factor infertility result from quantitative deficiencies in spermatogenesis, aberrations in spermatozoal ultramorphology, or defects in spermatozoal nuclear/DNA health. When intracytoplasmic sperm injection (ICSI) is the only sensible strategy to help effect pregnancy and live birth, rare circumstances occur when the question arises as to whether sperm from the testis may offer benefit over sperm in the ejaculate. In most cases, if ejaculated spermatozoa are available, testicular sperm would not be necessary. Comparison studies, therefore, that address this dilemma are not all "randomized" but simply relate and contrast harvested sperm and ejaculated sperm in regards to a number of outcome variables. This chapter will try to determine if there are clear situations or indications to employ retrieved testicular spermatozoa in place of available ejaculated spermatozoa in the couple undergoing ICSI to maximize their chances of a pregnancy and healthy offspring. To this end, a series of possible scenarios will be investigated.

R.D. Oates, M.D. (✉)
Department of Urology, Boston University School of Medicine and Boston Medical Center, 725 Albany Street, Suite 3B, Boston, MA 02118, USA
e-mail: robert.oates@bmc.org

2.2 Is Testis Sperm as Genetically Safe and Competent as Ejaculated Sperm and Vice Versa When Used as the Sperm Source for Intracytoplasmic Sperm Injection?

Before deciding upon whether testis sperm should be harvested in certain situations when ejaculated sperm are available, it may be helpful to look at the general data when testis sperm was used in cases of azoospermia and ejaculate sperm was used in cases of severe oligospermia. Was one better than the other—a bidirectional question?

It is known that ultimate ICSI success seems lower in men with spermatogenic compromise as compared to men with normal spermatogenesis [1–3]. Furthermore, Amirjannati et al. compared ICSI outcomes in isolated cases of severe spermatogenic compromise (cryptozoospermia—ejaculate sperm vs. nonobstructive azoospermia—testis sperm) and concluded that there was no difference in fertilization rate or embryo quality [4]. If spermatozoa were found, they were used as the sperm source. This is a similar conclusion to that of Bendikson et al. [5]. To shed some light on events at both the beginning and at the much later stages of a long continuum, Tsai et al. specifically compared the clinical and developmental outcomes in cases where either ejaculated sperm from men with extreme oligo-astheno-teratospermia (OAT) or surgically retrieved testicular sperm from men with azoospermia was used as the sperm source for ICSI

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_2,

[6]. No DNA fragmentation assays were performed beforehand, nor any other "selection" type testing was applied to move the investigators from use of ejaculate sperm in any individual to testis sperm. In a way then, this is a raw comparison of the two sperm sources in the absence of any biological/genetic characterization of the ejaculate sperm other than it was available and useful. Results showed no difference in rates of fertilization, number of embryos generated, embryo implantation rate, clinical pregnancy rate per embryo transfer, live birth rate, or miscarriage rate. Rates of congenital anomalies and developmental disorders were the same between the two groups. So even without "pre-ICSI testing" of the sperm in any other way than just making sure that viable, morphologically adequate sperm could be retrieved from the ejaculate of men with severe OAT, rates of success in every measurable parameter and rates of congenital/developmental anomalies were the same. Their data suggest that the ejaculate sperm of men with severe OAT and harvested testis sperm have the same potential vis-à-vis ICSI outcome and offspring health and that there may not be an overall benefit of moving from ejaculate to testis as the source. Of course, this is not a comparison within an individual but more a comparison between groups of men, depending upon sperm source, which is encouraging and informative.

Fedder et al. expanded upon these findings in a comprehensive study of their own. The authors compared the neonatal outcome of 8,967 children born via ICSI with ejaculated sperm, 17,592 children born via IVF with ejaculated sperm, and 63,854 children born via natural conception (the three control groups) with 466 children born after the use of harvested sperm from the epididymis or testis coupled with ICSI [7]. No testing was performed on ejaculate sperm that would have led the investigators to employ testis sperm instead [8]. When isolating results from ICSI with ejaculated sperm and ICSI with testis/epididymal sperm, there was no statistical difference in the sex ratio, mean birth weight for singletons, mean gestational age, rate of stillbirths, perinatal and neonatal mortality, congenital anomalies, or cardiac malformations. Studies supporting these data, especially as they relate to congenital and cardiac abnormalities, are few. However, Belva et al. speak to this point and concluded in their study comparing 530 children conceived with testis sperm and ICSI, 194 children conceived with epididymal sperm and ICSI, and 2,516 children conceived with ejaculated sperm and ICSI, "Overall neonatal health in terms of birth parameters, major anomalies, and chromosomal aberrations in our large cohort of children born by the use of non-ejaculated sperm seems reassuring in comparison to the outcome of children born after the use of ejaculated sperm" [9]. The authors are looking at their data with a question of whether testis sperm is as safe as ejaculate sperm, but it may also be concluded that ejaculate sperm is as safe as testis sperm. Parenthetically, in their elegant review, Pinborg et al. do show that there is a slightly higher congenital anomaly rate in babies born after ART, but conclude that it is difficult to identify the reasons behind that [10]. Additionally, Belva et al. have also shown that there is reassuring normal sexual maturation and pubertal development in a cohort of adolescent boys born to fathers via ICSI with male factor infertility (while not explicitly stated, this group of men probably was comprised of men with both severe oligo and nonobstructive azoospermics) [11]. Finally, Woldringh et al. concluded that there is no increased anomaly rate in children born after the use of nonejaculated sperm (testis and epididymal) as compared to ejaculated sperm [12, 13]. These data, similar to those cited above, were really comparing testis sperm (the variable) with ejaculate sperm (the control), but for the purposes of this chapter, the reverse can be inferred as well—there was no increased risk in the use of ejaculate sperm when compared to testis sperm. Again, these data suggest that as a general approach, there is no benefit in terms of ICSI success or offspring health of using testis sperm instead of ejaculate sperm or ejaculate sperm in preference to testis sperm—when viable, morphologically normal ejaculate sperm is available.

2.3 Is Testis Sperm the Answer When There Are Gross Morphological Abnormalities Seen In the Ejaculate Sperm?

There may be a temptation to extract testis sperm in hopes that it is morphologically superior to what is present in the ejaculate when the spermatozoa have severe and extremely abnormal morphological aberrations [14]. This may not necessarily be advantageous as there are so many components of the sperm head, neck, and midpiece (e.g., the centrosome), which are critically important for fertilization and the early stages of embryo development, beautifully reviewed by Schatten and Sun (centrosomes and centrosomal pathology) [15] and Chemes and Sedo (general sperm morphological pathologies) [16]. For example, globozoospermia, also known as round-headed sperm syndrome, is a condition in which the acrosomal cap does not form properly and, as a consequence, the sperm head assumes a spherical shape (seen as "round" on profile under the microscope). It occurs rarely (<1 % of the infertile male population), and men are otherwise phenotypically normal [17]. It is easily recognized upon formal semen analysis. These sperm lack the ability to fertilize, resulting in the infertility the couple experiences, but, in general, the sperm density and motility are adequate. There are three reported genetic etiologies. A homozygous deletion of *DPY19L2* has been described by Harbuz et al. and Koscinski et al. [18, 19]. *DPY19L2* is located on chromosome 12 and is expressed in the testis and must be necessary for proper acrosomal construction during spermiogenesis. Homozygous mutations in *SPATA16* and *PICK1* have also been described [20, 21]. There have been ICSI pregnancies reported using ejaculate globozoospermic spermatozoa. The importance of realizing that there is a well-known genetic basis predicts that the spermatozoa found at their origins in the seminiferous epithelium will be no different—better or worse—than the spermatozoa found floating in the ejaculate. This is a spermatozoal developmental disorder and not a morphological abnormality "acquired" after the sperm leave the protected confines of the seminiferous tubules.

A similar situation exists for spermatozoa affected by dysplasia of the fibrous sheath. The sperm have stubby, truncated, malformed tails, resulting from hypertrophy and hyperplasia of the fibrous sheath, abnormal midpiece assembly, and absent or malpositioned mitochondria [22]. Although the genetic basis has not yet been fully elucidated, this is, as above, a micro-developmental abnormality of sperm morphogenesis which occurs during the latter stages of spermatogenesis and the sperm derived by testicular extraction will offer no advantage to the patient [23] over that obtained in the ejaculate, unless there is no observable motility (vide infra).

Likewise, macrocephalic sperm head syndrome, a rare anomaly but one easily recognized on light microscopy (large irregular heads, abnormal midpieces, and multiple tails), is another developmental disorder in which one of the most important aspects is the polyploidy of the nucleus [24, 25]. Homozygous mutations of the aurora kinase C gene (*AURKC*) have been described [26]. When faced with these bizarre sperm in the ejaculate, there will be no advantage to harvesting testis sperm—this is not an acquired defect during spermatozoal transport.

2.4 Is Testis Sperm the Answer When There Is Extremely Limited Motility In the Ejaculate Sperm?

When assessing a semen sample's suitability for ICSI, motility is used as an appropriate surrogate for viability. Even if the sperm is just twitching (as it might be when derived from testis tissue), it must be viable. But is sperm in the ejaculate that is "just barely twitching" still functionally competent? If that sperm were completely normal in all respects, why would it be "just barely twitching" when drifting along in the ejaculate fluid? There are a few circumstances when testis sperm may be a better choice than this type of ejaculate sperm: subsequent to microsurgical ductal reconstruction (vasovasostomy or vasoepididymostomy), when the patient has had long-standing diabetes mellitus, and in cases of primary ciliary dyskinesia.

Occasionally, a post-reconstruction semen analysis will show excellent counts and motilities but then, over time, show a significant and steady drop in motility—occasionally to 0 %—due to anastomotic stricture formation [27, 28]. The sperm that eventually make their way into the ejaculate through the partial blockage at the site of the reconstruction may be senescent and not as active and capable as they once were. The key is the morphology of the entire sperm group within the sample—the tail of deceased sperm degenerate first (the reason that sperm heads without tails are often found in fluid proximal to the obstructed point) and so many of the sperm in these types of stricture cases show partial tails or no tails at all. This is a clue as to the nature of the cohort of sperm in the ejaculate—it is an admixture of spermatozoa that have finally been pushed through an anastomotic stricture and are aged, dead, or dying. In this circumstance, sperm that are barely twitching are not equivalent to those barely twitching sperm harvested from testis tissue, which are young and healthy but just have not yet gained the capacity for vigorous, progressive motility. These two types of trembling cells are on the opposite ends of the sperm life spectrum. If it is a difficult task to find visibly viable sperm for use as the sperm source for ICSI in these types of cases, the use of harvested testis sperm may be appropriate [29]. The same holds true for men with longstanding diabetes mellitus [30–32]. Due to micro-neuropathic and vascular disease, the vasa and seminal vesicles become dysfunctional, to the point in some men where they do not contract at all leading to failure of emission. Prior to complete failure, however, poorly motile and aged sperm may be found in the low volume ejaculate. If the sperm found in the seminal fluid are particularly deficient in motility and forward progression and oral alpha-sympathomimetic agents do not result in any improvement, testis sperm may be a better source of spermatozoa for ICSI. Even though alternative mechanisms to explain the infertility seen in some diabetic men have been postulated, the anatomical changes and peristaltic deficiency in the ductal system must be kept in mind [33]. The choice to pursue harvesting testis sperm must be based upon individual considerations as there are limited data addressing the above clinical situations.

Immotile cilia syndromes, aka primary ciliary dyskinesia, come in a variety of forms but, in many, spermatozoa have an absolute lack of motility [34]. Kartagener Syndrome is one such subtype. Spermatozoal axonemes display various types of ultrastructural defects, typically involving the inner and outer dynein arms [35]. Three of the ten genes that have definitely been implicated include: DNAI1 (chromosome 9p), DNAH5 (chromosome 5p), and DNAH11 (chromosome 7p) [36–39]. However, there may be well over 300 potential candidate genes related to cilia and, possibly, the ciliopathies [40]. Pregnancies have been reported with both ejaculate sperm (viability determined by hypoosmotic swelling) and testis sperm [41–44]. Therefore, since this is a genetic aberration that, essentially, limits the determination of viability by associating it with motility, either ejaculate sperm can be chosen for ICSI by a surrogate viability assessment (hypoosmotic swelling) or testis sperm can be utilized—both should have the same potential [45]. Most importantly, the patient should have the correct diagnosis made based upon the phenotypic characteristics of the disorder such as sinusitis, bronchiectasis, and situs inversus. This concept holds true for sperm afflicted with dysplasia of the fibrous sheath—if no motility can be observed in the deformed ejaculate sperm, testis sperm may offer an advantage as the chance of actually choosing viable sperm would likely be higher.

2.5 Is Testis Sperm the Answer When There Is Increased DNA Fragmentation In the Ejaculate Sperm or Repeated ICSI Failure for Unknown Reasons?

Sperm DNA damage can be measured in several different ways utilizing the Sperm Chromatin Structure Assay (SCSA) [46], the single-cell gel electrophoresis assay (COMET) [47], the Sperm Chromatin Dispersion test (SCD) [48], and the

deoxynucleotidyl Transferase-mediated dUTP Nick End Labeling assay (TUNEL) [49], as reviewed by Tamburrino et al. [50]. There exist abundant data supporting an association between spermatozoal DNA damage and fertility outcomes but not to the level where they, as a group or as individual tests, are useful prior to intervention [50, 51]. Although in agreement with that statement, Collins et al. do wonder whether there are subgroups of infertile couples that may indeed derive clinical and prognostic benefit from DNA integrity testing [52]. However, the question to be addressed here is twofold. First, is there evidence that sperm DNA damage affects the outcome of ICSI, when ICSI is the only treatment strategy available, as would be the case for severe oligospermia or nonobstructive azoospermia? Second, would the use of testicular sperm be advantageous in the circumstance when the ejaculate sperm is shown to have a high level of DNA damage? In regards to the first query, Zini et al. performed an elegant systematic review looking at studies that evaluated sperm DNA damage and embryo quality and development after IVF or ICSI [53]. They concluded that there is "no consistent relationship between sperm DNA damage and embryo quality and/or development". This probably has many reasons, of which one is the oocyte's ability, limited to a degree, for repair and restoration of damaged spermatozoal DNA (reviewed by Menezo et al. [54]). In regards to the second inquiry, Sakkas and Alvarez detail the many mechanisms and locations that DNA damage may occur, including several that are post-testicular [55]. Therefore, would testis sperm, in certain cases, provide "less-damaged" sperm for ICSI? Moskovtsev et al. do demonstrate that in men who showed no decrease in the levels of ejaculate DNA damage following oral antioxidant therapy, "retrieved testicular sperm had a lower degree of DNA damage compared with ejaculate sperm collected on the same day" [56]. Counter to this putative reason to harvest testis sperm in these cases, however, is the observation that testicular sperm may have a higher incidence of chromosomal anomalies than ejaculate sperm [57]. This was also seen in men who had high sperm DNA damage in simultaneous assessment of both their testicular and ejaculate sperm [58]. So, in referring to their own ongoing work, Moskovtsev et al. succinctly caution, "as TESE may be an invasive and expansive procedure, it should not be standard of care for patients with high sperm DNA damage until the randomized controlled trial has shown clear benefits in terms of pregnancy rates for these couples" [56].

Finally, even though there may be increased DNA fragmentation of ejaculate sperm in some men following vasectomy reversal, it has no prognostic value in terms of predicting pregnancy and so a move to harvest testis tissue if ejaculate sperm were available would not be indicated [59]. For those couples with repeated implantation failure, there is limited data available that supports a more invasive approach of harvesting testicular sperm in lieu of ejaculate sperm and some accumulating data suggesting that perhaps using the intracytoplasmic morphologically selected sperm injection (IMSI) technique may improve results [60, 61].

2.6 Conclusion

There is little evidence to support the contention that testis sperm may be a better gamete choice for ICSI than ejaculate sperm. This holds true in a variety of plausible circumstances but there is a paucity of data for very specific, perhaps individualized, situations. For example, in those men who have strictured anastomoses after microsurgical ductal reconstruction and just a few barely visibly viable sperm in a morass of degenerated and decaying spermatozoa, it may be better to harvest fresh and capable testis sperm. In those many cases of unexplained fertilization failure, poor embryo morphology, or deficient embryo implantation, future studies may provide some direction and information on which couples, if any, will benefit from changing the sperm source from ejaculate to testis. But for now, couples must be informed that a move to testis may be empirical and not a guaranteed solution.

References

1. Lee SH, Song H, Park YS, et al. Poor sperm quality affects clinical outcomes of intracytoplasmic sperm injection in fresh and subsequent frozen-thawed cycles: potential paternal effects on pregnancy outcomes. Fertil Steril. 2009;91(3):798–804.
2. Lu YH, Gao HJ, Li BJ, et al. Different sperm sources and parameters can influence intracytoplasmic sperm injection outcomes before embryo implantation. J Zhejiang Univ Sci B. 2012;13(1):1–10.
3. Naru T, Sulaiman MN, Kidwai A, et al. Intracytoplasmic sperm injection outcome using ejaculated sperm and retrieved sperm in azoospermic men. Urol J. 2008; 5(2):106–10.
4. Amirjannati N, Heidari-Vala H, Akhondi MA, et al. Comparison of intracytoplasmic sperm injection outcomes between spermatozoa retrieved from testicular biopsy and from ejaculation in cryptozoospermic men. Andrologia. 2012;44 (Suppl 1):704–9.
5. Bendikson KA, Neri QV, Takeuchi T, et al. The outcome of intracytoplasmic sperm injection using occasional spermatozoa in the ejaculate of men with spermatogenic failure. J Urol. 2008;180(3):1060–4.
6. Tsai CC, Huang FJ, Wang LJ, et al. Clinical outcomes and development of children born after intracytoplasmic sperm injection (ICSI) using extracted testicular sperm or ejaculated extreme severe oligo-asthenoteratozoospermia sperm: a comparative study. Fertil Steril. 2011;96(3):567–71.
7. Fedder J, Loft A, Parner ET, et al. Neonatal outcome and congenital malformations in children born after ICSI with testicular or epididymal sperm: a controlled national cohort study. Hum Reprod. 2013;28(1):230–40.
8. Fedder J. Personal Communication. In: Oats R, editor.2013.
9. Belva F, De Schrijver F, Tournaye H, et al. Neonatal outcome of 724 children born after ICSI using non-ejaculated sperm. Hum Reprod. 2011;26(7):1752–8.
10. Pinborg A, Henningsen AK, Malchau SS, et al. Congenital anomalies after assisted reproductive technology. Fertil Steril. 2013;99:327–32.
11. Belva F, Roelants M, Painter R, et al. Pubertal development in ICSI children. Hum Reprod. 2012;27(4): 1156–61.
12. Woldringh GH, Besselink DE, Tillema AH, et al. Karyotyping, congenital anomalies and follow-up of children after intracytoplasmic sperm injection with non-ejaculated sperm: a systematic review. Hum Reprod Update. 2010;16(1):12–9.
13. Woldringh GH, Horvers M, Janssen AJ, et al. Follow-up of children born after ICSI with epididymal spermatozoa. Hum Reprod. 2011;26(7):1759–67.
14. Harnisch B, Oates R. Genetic disorders related to male factor infertility and their adverse consequences. Semin Reprod Med. 2012;30(2):105–15.
15. Schatten H, Sun QY. New insights into the role of centrosomes in mammalian fertilization and implications for ART. Reproduction. 2011;142(6):793–801.
16. Chemes HE, Alvarez Sedo C. Tales of the tail and sperm head aches: changing concepts on the prognostic significance of sperm pathologies affecting the head, neck and tail. Asian J Androl. 2012;14(1):14–23.
17. Dam AH, Feenstra I, Westphal JR, et al. Globozoospermia revisited. Hum Reprod Update. 2007;13(1):63–75.
18. Harbuz R, Zouari R, Pierre V, et al. A recurrent deletion of DPY19L2 causes infertility in man by blocking sperm head elongation and acrosome formation. Am J Hum Genet. 2011;88(3):351–61.
19. Koscinski I, Elinati E, Fossard C, et al. DPY19L2 deletion as a major cause of globozoospermia. Am J Hum Genet. 2011;88(3):344–50.
20. Dam AH, Koscinski I, Kremer JA, et al. Homozygous mutation in SPATA16 is associated with male infertility in human globozoospermia. Am J Hum Genet. 2007;81(4):813–20.
21. Liu G, Shi QW, Lu GX. A newly discovered mutation in PICK1 in a human with globozoospermia. Asian J Androl. 2010;12(4):556–60.
22. Moretti E, Geminiani M, Terzuoli G, et al. Two cases of sperm immotility: a mosaic of flagellar alterations related to dysplasia of the fibrous sheath and abnormalities of head-neck attachment. Fertil Steril. 2011;95(5):1787. e19–23.
23. Chemes HE, Rawe VY. The making of abnormal spermatozoa: cellular and molecular mechanisms underlying pathological spermiogenesis. Cell Tissue Res. 2010;341(3):349–57.
24. Molinari E, Mirabelli M, Raimondo S, et al. Sperm macrocephaly syndrome in a patient without AURKC mutations and with a history of recurrent miscarriage. Reprod Biomed Online. 2012;26:148–56.
25. Shimizu Y, Kiumura F, Kaku S, et al. Successful delivery following ICSI with macrocephalic sperm head syndrome: a case report. Reprod Biomed Online. 2012;24(6):603–5.
26. Dieterich K, Soto Rifo R, Faure AK, et al. Homozygous mutation of AURKC yields large-headed polyploid spermatozoa and causes male infertility. Nat Genet. 2007;39(5):661–5.
27. Carbone Jr DJ, Shah A, Thomas Jr AJ, et al. Partial obstruction, not antisperm antibodies, causing infertility after vasovasostomy. J Urol. 1998;159(3): 827–30.
28. Jarow JP, Sigman M, Buch JP, et al. Delayed appearance of sperm after end-to-side vasoepididymostomy. J Urol. 1995;153(4):1156–8.
29. Chavez-Badiola A, Drakeley AJ, Finney V, et al. Necrospermia, antisperm antibodies, and vasectomy. Fertil Steril. 2008;89(3):723. e5–7.
30. La Vignera S, Calogero AE, Condorelli R, et al. Andrological characterization of the patient with diabetes mellitus. Minerva Endocrinol. 2009;34(1):1–9.
31. La Vignera S, Condorelli R, Vicari E, et al. Diabetes mellitus and sperm parameters. J Androl. 2012;33(2): 145–53.
32. La Vignera S, Vicari E, Condorelli R, et al. Ultrasound characterization of the seminal vesicles in infertile

patients with type 2 diabetes mellitus. Eur J Radiol. 2011;80(2):e64–7.
33. Roessner C, Paasch U, Kratzsch J, et al. Sperm apoptosis signalling in diabetic men. Reprod Biomed Online. 2012;25(3):292–9.
34. Leigh MW, Pittman JE, Carson JL, et al. Clinical and genetic aspects of primary ciliary dyskinesia/Kartagener syndrome. Genet Med. 2009;11(7):473–87.
35. Kawakami M, Hattori Y, Nakamura S. Reflection of structural abnormality in the axoneme of respiratory cilia in the clinical features of immotile cilia syndrome. Intern Med. 1996;35(8):617–23.
36. Chodhari R, Mitchison HM, Meeks M. Cilia, primary ciliary dyskinesia and molecular genetics. Paediatr Respir Rev. 2004;5(1):69–76.
37. Collodel G, Moretti E. Sperm morphology and aneuploidies: defects of supposed genetic origin. Andrologia. 2006;38(6):208–15.
38. Moretti E, Collodel G. Three cases of genetic defects affecting sperm tail: a FISH study. J Submicrosc Cytol Pathol. 2006;38(2–3):137–41.
39. Rives N, Mousset-Simeon N, Mazurier S, et al. Primary flagellar abnormality is associated with an increased rate of spermatozoa aneuploidy. J Androl. 2005;26(1):61–9.
40. Geremek M, Bruinenberg M, Zietkiewicz E, et al. Gene expression studies in cells from primary ciliary dyskinesia patients identify 208 potential ciliary genes. Hum Genet. 2011;129(3):283–93.
41. Cayan S, Conaghan J, Schriock ED, et al. Birth after intracytoplasmic sperm injection with use of testicular sperm from men with Kartagener/immotile cilia syndrome. Fertil Steril. 2001;76(3):612–4.
42. Kaushal M, Baxi A. Birth after intracytoplasmic sperm injection with use of testicular sperm from men with Kartagener or immotile cilia syndrome. Fertil Steril. 2007;88(2):497. e9–11.
43. Papadimas J, Tarlatzis BC, Bili H, et al. Therapeutic approach of immotile cilia syndrome by intracytoplasmic sperm injection: a case report. Fertil Steril. 1997;67(3):562–5.
44. Peeraer K, Nijs M, Raick D, et al. Pregnancy after ICSI with ejaculated immotile spermatozoa from a patient with immotile cilia syndrome: a case report and review of the literature. Reprod Biomed Online. 2004;9(6):659–63.
45. McLachlan RI, Ishikawa T, Osianlis T, et al. Normal live birth after testicular sperm extraction and intracytoplasmic sperm injection in variant primary ciliary dyskinesia with completely immotile sperm and structurally abnormal sperm tails. Fertil Steril. 2012; 97(2):313–8.
46. Evenson DP, Jost LK, Marshall D, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod. 1999;14(4):1039–49.
47. Singh NP, McCoy MT, Tice RR, et al. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. 1988;175(1):184–91.
48. Fernandez JL, Muriel L, Rivero MT, et al. The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. J Androl. 2003;24(1):59–66.
49. Sun JG, Jurisicova A, Casper RF. Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. Biol Reprod. 1997;56(3):602–7.
50. Tamburrino L, Marchiani S, Montoya M, et al. Mechanisms and clinical correlates of sperm DNA damage. Asian J Androl. 2012;14(1):24–31.
51. The clinical utility of sperm DNA integrity testing. Fertil Steril. 2008 Nov;90(5 Suppl):S178–80.
52. Collins JA, Barnhart KT, Schlegel PN. Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? Fertil Steril. 2008;89(4):823–31.
53. Zini A, Jamal W, Cowan L, et al. Is sperm DNA damage associated with IVF embryo quality? A systematic review. J Assist Reprod Genet. 2011;28(5):391–7.
54. Menezo Y, Dale B, Cohen M. DNA damage and repair in human oocytes and embryos: a review. Zygote. 2010;18(4):357–65.
55. Sakkas D, Alvarez JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril. 2010;93(4):1027–36.
56. Moskovtsev SI, Jarvi K, Mullen JB, et al. Testicular spermatozoa have statistically significantly lower DNA damage compared with ejaculated spermatozoa in patients with unsuccessful oral antioxidant treatment. Fertil Steril. 2010;93(4):1142–6.
57. Rodrigo L, Rubio C, Peinado V, et al. Testicular sperm from patients with obstructive and nonobstructive azoospermia: aneuploidy risk and reproductive prognosis using testicular sperm from fertile donors as control samples. Fertil Steril. 2011;95(3):1005–12.
58. Moskovtsev SI, Alladin N, Lo KC, et al. A comparison of ejaculated and testicular spermatozoa aneuploidy rates in patients with high sperm DNA damage. Syst Biol Reprod Med. 2012;58(3):142–8.
59. Smit M, Wissenburg OG, Romijn JC, et al. Increased sperm DNA fragmentation in patients with vasectomy reversal has no prognostic value for pregnancy rate. J Urol. 2010;183(2):662–5.
60. Knez K, Zorn B, Tomazevic T, et al. The IMSI procedure improves poor embryo development in the same infertile couples with poor semen quality: a comparative prospective randomized study. Reprod Biol Endocrinol. 2011;9:123.
61. Weissman A, Horowitz E, Ravhon A, et al. Pregnancies and live births following ICSI with testicular spermatozoa after repeated implantation failure using ejaculated spermatozoa. Reprod Biomed Online. 2008;17(5): 605–9.

The Aging Male and Impact on Offspring

3

Timothy G. Jenkins, Kenneth I. Aston, and Douglas T. Carrell

3.1 Introduction

Advanced paternal age has become a heavily investigated topic recently as a result of multiple studies demonstrating ties between advanced paternal age and various offspring abnormalities. Further contributing to the increasing interest in the role of advanced paternal age in reproduction is the trend of delayed parentage believed to be a result of socioeconomic pressures in developed countries [1]. Though this trend is justified by increasing life expectancies in both sexes, advanced paternal age significantly affects general semen parameters and sperm quality that ultimately alters fecundity and may additionally affect offspring health. While many couples consider the risks associated with advanced maternal age in family planning decisions, very little thought is given to the age of male partners. As a result, it is important that physicians consulting couples with an aged male partner have the available data to help patients make well-informed family planning decisions based on the risks associated with advanced paternal age. This chapter will outline what is currently known regarding the effects of paternal age on fecundity and will also discuss the associations between advanced paternal age and the offspring's disease risk. These effects, based on current data are summarized in Table 3.1.

T.G. Jenkins, B.S.
Andrology and IVF Laboratories, Department of Surgery, University of Utah School of Medicine, Salt Lake City, UT, USA

K.I. Aston, Ph.D., H.C.L.D.
Andrology and IVF Laboratories, Division of Urology, Department of Surgery, University of Utah School of Medicine, Salt Lake City, UT, USA

D.T. Carrell, Ph.D., H.C.L.D. (✉)
Andrology and IVF Laboratories, Division of Urology, Department of Surgery, University of Utah School of Medicine, Salt Lake City, UT, USA

Department of Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, UT, USA

Department of Human Genetics, University of Utah School of Medicine, 675 Arapeen Dr. Suite 201, Salt Lake City 84108, UT, USA
e-mail: douglas.carrell@hsc.utah.edu

3.2 Delayed Parenthood

In recent history, the age of parenthood for both males and females has steadily increased in many developed countries. This trend is believed to be associated with increased life expectancy, socioeconomic pressures, and divorce rates with subsequent remarriage at older ages [2]. During a 10-year span (1993–2003) in Great Britain, the percent of fathers who were in the age range of 35–54 increased from 25 % of total births to 40 %. Associated with this trend was a decrease in the number of births to fathers less than 35 years of age from 74 % of total births to only 60 % [3]. In Australia, over two decades (1988–2008), the

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_3,

Table 3.1 The effects of advanced paternal age on semen parameters and offspring disease risk

Parameter	Effect
Semen parameters	
Semen volume	↓
Sperm count	↓?
Sperm motility	↓
Sperm morphology	↓
Genetic/epigenetic	
DNA damage	↑
Aneuploidy rates	
Sex chromosomes	↑
Autosomes	~
Mutations	↑
Telomere length	↑
Chromatin packaging	Δ
Global methylation	Δ
Pregnancy rate	
Natural conception	↓
Insemenation	↓?
IVF	↓?
Offspring disease risk	
Autosomal dominant disorders	↑
Trinucleotide repeat disorders	↑
Cancer	
Hematologic	↑
Brain tumors	↑
Breast	↑
Prostate	↑
Neuropsychiatric disorders	↑

↓, decline; ↑, increase; Δ, change; ~, no change; ?, data are ambiguous

average age of fathers has increased by approximately 3 years [4]. Similarly, the average age of fathers in Germany increased by 2 years over a 10-year period [2]. Similar trends can be found in the United States and many other developed countries. As average paternal age continues to increase in many countries it is becoming increasingly important to characterize the potential consequences of advanced paternal age on fertility and offspring health.

3.3 Age-Related Changes in Sperm Quality

With advancing male age, a number of changes occur to sperm and semen that can impact fertility status or increase the risk of disease transmission to offspring. These changes include declines in some semen parameters, increased sperm DNA damage, genetic changes in sperm resulting from mitotic or meiotic errors or errors that arise during DNA replication, and epigenetic changes to sperm DNA. These changes are discussed below.

3.3.1 Changes in Semen Parameters

Unlike females, who are born with a finite number of gametes that are generally exhausted between the age of 45 and 55 years, coincident with menopause, men continue to produce sperm throughout their lives. While spermatogenesis continues well into old age, some semen parameters do decline as men age. Numerous studies have evaluated the effects of male age on semen parameters, but shortcomings of some of the individual studies include small sample size and failure to control for potentially confounding factors. For this reason there exists a significant degree of discordance between studies, making the reliable estimate of age effects difficult to quantify. However, a thorough review of the literature from 1980 to 1999 by Kidd et al. evaluated the effect of age on semen parameters and concluded that there is general agreement among studies that semen volume, sperm motility, and proportion of morphologically normal sperm all decline with advancing age [5]. These conclusions were corroborated by more recent literature reviews and carefully controlled primary research [6–8].

From the available literature, it can be inferred that semen volume significantly decreases with age, with a decline of 3–22 % from age 30 to age 50 [5, 8]. Similarly, a 3–37 % decrease in sperm motility is estimated to occur over the same period, as indicated in several studies [5, 8]. Finally, the best estimates for declines in normal sperm morphology indicate a decrease of 4–22 % between the ages of 30 and 50 [5, 8]. The data regarding changes in sperm concentration with age are less conclusive, and total sperm count has rarely been evaluated. Of more than 20 studies that evaluated the effect of male age on sperm concentration, there is essentially an even split between studies that report a decline, those that report no age effect, and those that report increased

sperm concentrations with advancing age [5, 8]. As semen volume significantly declines with age, if spermatogenic output remained constant, then sperm concentration would necessarily increase in older men. A recent study of 1,174 men age 45 and older reported a non-significant increase in sperm concentration with age, and a significant decline in total sperm count with advancing age in men between the ages of 45 and 80 [9].

While the consensus based on large datasets is that semen volume, sperm motility, and normal sperm morphology decrease with advancing age, the decreases are generally modest. Moreover, the number of confounding variables such as lifestyle factors, environmental influences, health status, abstinence periods, and others make it nearly impossible to identify the age-associated causes that are directly responsible for these declines.

3.3.2 Genetic Changes

The molecular hallmarks of aging throughout the body include increased oxidative damage, increased aneuploidy rates and chromosomal rearrangements, the accumulation of mutations within the genome, and telomere shortening [10, 11]. Sperm are particularly prone to many of these changes due to the high rate of cell division relative to most other cells types in the body. However, unlike telomere attrition that occurs in the majority of other cell types, the telomeres length in sperm actually increases with age. Genetic changes to sperm are discussed in the following section.

3.3.2.1 DNA Damage

Numerous studies have reported an age-related increase in sperm DNA damage [12–16]. The increase in DNA fragmentation index (DFI) is marked, with a nearly fourfold increase in men age 60–80 compared with men age 20–29 reported in one study [14]. In a large study of 1,125 men from infertile couples, DFI more than doubled in men over the age of 45 compared with men aged 30 and younger [16]. The mechanisms responsible for increased sperm DNA damage in older men are not completely characterized, but increased reactive oxygen species (ROS) [17], coupled with the insufficiency of DNA repair and apoptotic machinery, have been proposed [18].

3.3.2.2 Aneuploidy Rates

The increase in gamete aneuploidy rates in women with advancing age is well documented and dramatic. It is estimated that about 20 % of human oocytes are aneuploid, and the incidence has been reported to be as high as 60 %, with a sharp increase in the decade preceding menopause [19–21]. In contrast, sperm aneuploidy rates are much lower with an estimated average incidence of 1–2 % [20], and the effect of male age on sperm aneuploidy rate remains unclear. Some studies have failed to find an effect of male age on sperm aneuploidy frequency [14, 22], while others have reported a modest increase in aneuploidy rates related to age, particularly increased disomies of the sex chromosomes [23–25].

While there is no consensus on the effect of male age on sperm aneuploidy rates, the majority of evidence suggests a slight increase in sex chromosome disomy rates in older men and a general lack of an effect or a weak effect in the autosomes [8].

3.3.2.3 Increased Mutations

The introduction of de novo mutations into the genome is the basis for heritable genetic variation, and the number of mutations per genome is related to the number of replication cycles that a cell undergoes, as there is an error rate inherent in replication machinery. Based on family-based sequencing and single sperm sequencing as well as evolutionary measures, the de novo mutation rate of sperm is estimated to be between 1 and 4 changes per 100 million bases per generation [26, 27], while the mutation rate per cell division is almost three orders of magnitude lower than the per generation mutation rate [28]. The more cycles of DNA replication and cell division a cell undergoes, the greater the chance for mutations to occur in that cell. In women, from the primordial germ cell stage to ovulation, an oocyte will have undergone approximately 24 cell divisions [29]. In men that number is estimated to be approximately 30 cell divisions at puberty, with one spermatocyte cell division every 16 days, or 23 divisions per year after puberty (see Fig. 3.1) [29].

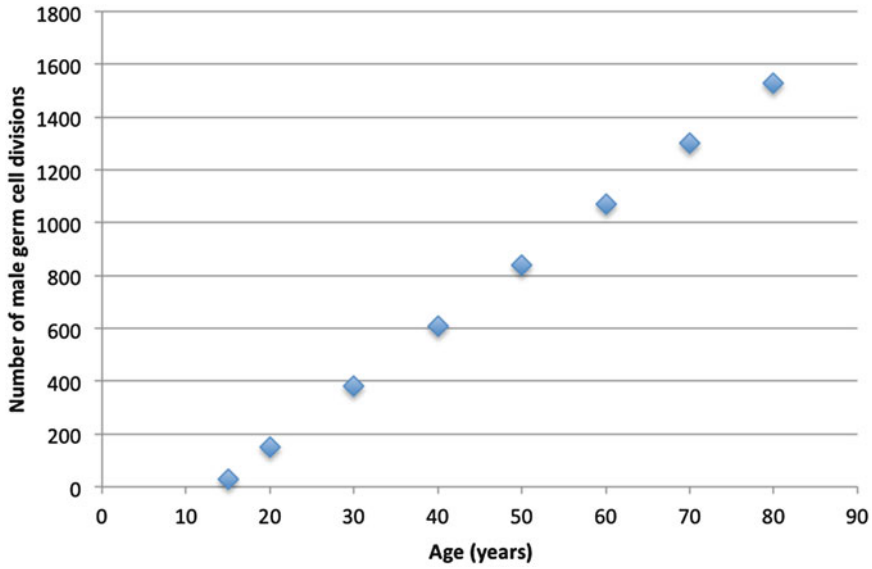

Fig. 3.1 Illustration of the estimated number of male germ cell divisions as a function of age

Clearly there is a greater opportunity for mutations to arise in sperm than in oocytes, and male age is predicted to be a strong contributing factor. Lionel Penrose was the first to propose a relationship between male age and mutations in offspring [30]. While the mutation load of individual sperm as a function of male age has not been directly measured, molecular genetics predicts that sperm from older men will, on average, harbor more mutations than sperm from younger men. This prediction is substantiated by a recent study of genomic sequence in parent–offspring trios that estimated an increase of approximately two mutations per year of paternal age [31]. In addition, the increased rates of specific autosomal dominant diseases and disease-specific mutation analysis also support an age effect on sperm mutation frequency [14], as will be discussed in detail below.

3.3.2.4 Changes in Telomeres

While the consequences of advanced paternal age on the genetics of sperm are generally negative, the age-related changes to sperm telomeres might confer some advantage to offspring. Telomeres are composed of long tracts of TTAGGG repeats located at the ends of each chromosome and serve as a buffer to the loss of important genetic material due to the inability of DNA replication machinery to replicate DNA at the very end of each chromosome. In addition, the telomere cap at the end of each chromosome distinguishes chromosome ends from double strand breaks and thus serves to protect against spurious chromosomal fusion [32]. While in most tissues, telomeres progressively shorten with age, ultimately resulting in cell cycle arrest or apoptosis, the telomeres in sperm are longer in older men [33], and children of older fathers have longer leukocyte telomeres than do children of younger fathers [34, 35]. Telomere inheritance may represent an example of a genetic advantage of delayed reproduction in men as longer leukocyte telomere length is associated with decreased risk of atherosclerosis and increased lifespan [36].

3.3.3 Epigenetic Changes

The effect of advanced paternal age on offspring has begun to receive much attention. Recent studies have linked paternal aging and the prevalence of well-known neuropsychiatric disorders in offspring [37–39]. Large retrospective studies demonstrate the effect of paternal age on various birth outcomes, including weight, premature deliveries, and various offspring abnormalities

[40, 41]. Additionally, recent research has begun to elucidate associations between aged fathers and increased incidence of obesity in offspring. These findings were independent of maternal age and other outside factors [42]. However, the etiology of the increased frequency of various disorders in the offspring of aged males remains poorly defined, though there are likely candidates.

In both sexes, aging alters DNA methylation marks in most somatic tissues throughout the body [43, 44]. Because of its prevalence in other cell types, aging-associated DNA methylation alteration is likely to occur in sperm as well. In fact, Oakes et al. have described age-associated hypermethylation at specific genomic loci in both sperm and liver tissue in male rats [44]. Similarly, our laboratory has identified increased global DNA methylation associated with age in human sperm from fertile donors (unpublished data). In further support of this idea is work demonstrating that frequently dividing cells have more striking methylation changes associated with age than do cells which divide less often [45]. Additionally, a recent study also indicates that, at specific gene promoters, there is increased DNA methylation in the offspring of older fathers [46]. These data further suggest the possibility of heritable DNA methylation alterations associated with advanced paternal age.

In addition to DNA methylation alterations there are data to suggest alterations in chromatin packing that occur with age as well. It has been suggested that chromatin remodeling plays a key role in cellular senescence, organismal aging, and age-associated disease and thus could play a role in age-associated sperm alterations that may ultimately affect the offspring [47]. In fact, Nijs et al. described altered chromatin packing associated with age as assessed by the sperm chromatin structure assay [48]. The subtle nature of the effect and, in some cases, the absence of well-characterized genetic factors, in addition to the aging-associated somatic cell methylation alterations, suggest that a major contributing factor to the increased prevalence of various diseases among the offspring of aged fathers is the sperm epigenome.

3.4 Reproductive Consequences of Age-Related Changes in Sperm

3.4.1 Fecundity

Among the consequences of delayed paternity, and likely the most dramatic alteration that occurs with increased paternal age, is that of decreased fecundity. Though very different from the universal and abrupt age-associated cessation of fertility seen in females, there is a significant decline in a male's capacity to produce viable offspring that is correlated with age. However, the age at which an individual male's reproductive capacity declines and even the frequency of this decline among a population of men remains controversial. Despite this, there are many studies that demonstrate an age effect on male fecundity with study groups, including natural conception, artificial insemination, and in vitro fertilization.

In an observational study performed in the United Kingdom in 2003, Hassan et al. found that men >45 years of age had a fivefold increase in their time to pregnancy in comparison to individuals <25 years of age [49]. Interestingly, when compared to males <25, men 45 and older were also 12.5 times more likely to have a time to pregnancy of greater than 2 years [49]. As expected, this effect is amplified when the female member of a couple is of advanced reproductive age as well (35–39). In these couples, men >40 were more than two times more likely to fail to conceive during a 12 month period in comparison to men <40 [50]. Additionally, when taken into account unsuccessful pregnancies in the same groups men over 40 were three times less likely to produce viable offspring than do the younger cohort [50]. Other studies support these data by suggesting an increased frequency of fetal loss to those fathered by older men, increased time to pregnancy, and decreased probability of conception [51–53]. However, there are conflicting data which suggest little to no effect of paternal age on fertility in natural conception [54].

Research has also described effects of paternal age on the outcomes of assisted reproductive techniques. A total of 17,000 intrauterine insemination

(IUI) cycles analyzed in a French study revealed that the pregnancy rate for couples whose male partner was less than 30 years of age had a pregnancy rate of 12.3 % where couples whose male partner was over 30 years of age had a significantly lower pregnancy rate of 9.3 % after adjusting for female age [55]. Similarly, in 1995, Mathieu et al. showed that increasing male age (≥35 years of age) was associated with decreased rates of conception [56]. However, these data are controversial. Additional studies have failed to find a paternal age effect on IUI pregnancy rates [57]. Other studies have analyzed the paternal age effect on in vitro fertilization (IVF) success with a similar controversy. Many studies suggested that there is a paternal age effect in achieving viable pregnancy outcomes in IVF cycles [58] and also have suggested that this effect is amplified with partners of advanced maternal age [59]. In large studies involving the use of donor eggs in an IVF cycle showed a significant effect of paternal age on pregnancy outcome [60]. However, an even more recent study that corrected for age of the egg donor found no effect of paternal age on pregnancy outcome [61].

3.4.2 Disease Risk in Offspring

As would be expected, the numerous genetic and epigenetic changes that occur to sperm through the aging process are associated with elevated risk of some diseases in the offspring of older fathers. These include several rare, autosomal disorders, disorders involving expanded trinucleotide repeats, offspring aneuploidy, certain cancers, and several neuropsychiatric disorders. These diseases and associated risks will be discussed below. While risks of these disorders are demonstrably elevated in offspring of older fathers, it is important to emphasize that the paternal age contribution to the increased risk is generally quite low (with the exception of the autosomal dominant and triplet repeat disorders) and absolute risk for any of these disorders remains quite low.

3.4.2.1 Autosomal Dominant Disorders

Rare autosomal disorders, including Apert syndrome and achondroplasia, are among the most striking and earliest characterized examples of increased disease risk as a consequence of advanced paternal age. As early as 1912, it was observed that sporadic cases of achondroplasia, a dominantly inherited form of dwarfism, was most often found in the last-born children of a family [29]. More recently, a number of other diseases have been shown to display similar paternal age effects.

A dozen diseases showing a significant paternal age effect were described in a paper more than three decades ago, and several others have been described since that time [62]. In addition to achondroplasia and Apert syndrome, the list of autosomal dominant disorders that display a paternal age effect includes acrodysostosis, fibrodysplasia ossificans progressive, neurofibromatosis, multiple endocrine neoplasia 2A (MEN 2A) and MEN 2B, and syndromes including Marfan, Treacher-Collins, Crouzon, Noonan, and Pfeiffer, among others [62].

Remarkably, many of these conditions, including Apert syndrome, achondroplasia, Crouzon syndrome, Pfeiffer syndrome, MEN 2A, and MEN 2B, involve mutations in three genes, *FGF3R*, *FGFR2*, and *RET* [29, 63]. Moreover, in almost every case where parental origin of the de novo, disease-causing mutation in these genes was assessed, the mutation was paternally derived [29, 63–68]. In addition, the mutated loci linked to many of these disorders are among the most frequently mutated nucleotides in the entire genome [29]. These observations led to the hypothesis of selfish spermatogonial selection, the idea that some spermatogonial mutations confer some advantage, leading to clonal expansion of mutant sperm over time [63, 69]. This mechanism may explain, at least in part, the molecular basis for the increased incidence of these disorders with advanced paternal age.

While it is well established that increasing paternal age does increase the risk for numerous autosomal dominant disorders, it is important to note that the absolute risk for these diseases remains quite low. Additional research is required to fully characterize the mechanisms involved in increased transmission of these diseases by older fathers.

3.4.2.2 Trinucleotide Repeat Disorders

In addition to the association between point mutations in the male germline and male age, there is also evidence to suggest that other genomic changes, namely changes in trinucleotide repeat length, are also more frequent in the germline of older men. The cause of Huntington's disease has been traced to an expanded block of CAG tandem repeats within the Huntingtin (*HTT*) gene [70]. Longer triplet repeats in *HTT* result in altered protein function and Huntington's symptoms. It was demonstrated that repeat expansion is almost entirely driven through the male germline [71], and the extent of repeat expansion is significantly associated with paternal age [72].

Myotonic dystrophy (DM) is another disease associated with trinucleotide repeat expansion. Like Huntington's disease, expanded CTG repeats are more frequently transmitted from the father [73], and paternal age appears to be a risk factor for transmission of the disease [74]. One large study of 3,419 cases of Down syndrome did find a significant paternal age effect after adjusting for maternal age when mothers were older than 35, and the paternal age effect was most significant when maternal age was over 40 [75].

3.4.2.3 Offspring Aneuploidy

The majority of aneuploidies are embryonic lethal, however trisomies 13, 18, and 21 along with sex chromosome aneuploidies (XXY, XYY, XXX, XO, etc.) are compatible with life. The great majority of somatic aneuploidies are maternally derived. For example in a cohort of 352 cases of Down syndrome, approximately 91 % were of maternal origin, and a maternal contribution to other cases of trisomy involving chromosomes 13, 14, 15, and 22 were similar, ranging from 83 to 89 % [76]. Interestingly, the story is different for sex chromosome aneuploidies, with a little more than half of cases being paternally derived [20].

Given the relatively minor effect of paternal age on sperm aneuploidy rates, it is not surprising that epidemiologic data for the paternal contribution to trisomic offspring generally do not support a paternal age effect [8, 77, 78]. A recent study based on 22 EUROCAT congenital anomaly registers identified a marginally significant association between paternal age and Klinefelter syndrome [79]. Several studies have evaluated the relationship between paternal age and incidence of Down syndrome, and in general have reported a weak paternal age effect [80] or no effect at all [81]. Based on available data, clearly the paternal age effect on offspring aneuploidy is relatively small and is eclipsed by the significant maternal age effect.

3.4.2.4 Cancer

Based on the current literature, it appears that paternal age may have an effect on incidence of various types of cancers in offspring. These data are intriguing but remain quite controversial. One of the most heavily studied classes of disease in these studies is hematological cancers. A recent epidemiological study has described a decreased risk of acute myeloid leukemia in firstborn children, indirectly suggesting that maternal and paternal age may play a role in the frequency of cancer incidence in the offspring. The same study was able to directly detect an increased risk of being diagnosed with any form of childhood leukemia in children sired by fathers of between 35 and 45 years of age when compared to fathers <25 years of age [82]. In agreement with these data is research by Murray et al. which suggests that children born to fathers >35 years of age are 50 % more likely (relative risk = 1.5) to receive a diagnosis of a childhood leukemia [83]. However, a Swedish epidemiological study published in 1999 detected no significant impact of paternal age on hematologic cancers [84].

The impact of paternal age on offspring cancer incidence is not limited to hematologic metastases. There also appears to be an increased risk of developing childhood central nervous system tumors in the offspring of older fathers. One retrospective study showed that children born to a father >30 years of age were at a 25 % increased risk of developing a childhood brain tumor compared to children of fathers <25 years [84]. Similarly, Yip et al. demonstrated that the offspring of fathers >40 had an increased relative risk (approximately 1.7) of developing a central nervous system cancer [85].

Advanced paternal age also appears to affect the incidence of adult onset cancers in offspring.

The incidence of breast cancer has been shown to increase in the daughters of fathers who are >40 compared to fathers <30 [86]. Similarly, prostate cancer risk increases by approximately 70 % in the offspring of fathers >38 years of age compared to the children of fathers <27 years of age [87].

The mechanism behind this effect is likely multifactorial and may additionally vary by race. However, there are some candidates that likely play at least some role in the etiology of increased incidence of multiple cancers seen in the offspring of aged fathers. Environmental exposures that accumulate throughout the life of a male are one of the most likely effectors, as this may affect subtle DNA mutations and epigenetic alterations that are capable of being inherited. In fact, as mentioned earlier, there are some data that suggest that the offspring of older fathers have increased levels of DNA methylation at specific loci [46]. If any of these alterations (gene mutations or epigenetic modifications) occur at tumor suppressor genes or other important genes in the etiology of various cancers, the result would be increased cancer incidence as is seen in the current literature. Though this correlation is intriguing, it should be noted that much work is still required to further define the effects of paternal aging on the incidence of cancer in offspring.

3.4.2.5 Neuropsychiatric Disorders

In recent years, with the application of genomic tools, the genetic complexity of neuropsychiatric disorders is becoming increasingly apparent. However, it has long been suggested that advanced paternal age is a risk factor for schizophrenia [88], and more recently, advanced paternal age has been implicated in risk for autism, bipolar disorder, behavioral disorders, and reduced cognitive ability.

The paternal age effects on schizophrenia risk have been widely studied [89–91]. A recent meta-analysis representing 24 qualifying studies confirmed advanced paternal age to be a significant risk factor for schizophrenia [89]. In this study, the authors reported a slight but significant increase in the risk of developing schizophrenia in offspring from fathers >30 years of age, with relative risk (RR) increasing in older fathers. At the extreme, a combined RR for schizophrenia in the offspring of fathers >50 years of age compared with fathers age 25–29 was 1.66 [89]. Interestingly, there also appears to be a slight but significant risk of schizophrenia in offspring of fathers < 25 years (RR = 1.08) only in male offspring [89].

Associations between paternal age and risk of autism spectrum disorders (ASD) have also been thoroughly investigated, with two meta-analyses confirming a significant association [92, 93]. In the most recent population-based study and meta-analysis, it was estimated that fathers >50 years of age had a 2.2-fold increased risk of autism in offspring compared with men aged 29 years or less [93].

The data regarding the association between paternal age and other neuropsychiatric and behavioral disorders are less clear, but there does seem to be an increase in bipolar disorder [94, 95] and behavioral issues [96, 97] in children of older fathers. In addition, some studies indicate that children of older fathers display slightly reduced IQ compared with children of younger fathers [98, 99], although the differences are small, and conflicting reports exist [100].

While evidence clearly suggests that paternal age does have some impact on neurological development and the incidence of neuropsychiatric disorders, the mechanisms for neurodevelopmental changes have not been elucidated. It has been suggested that increased risk may be related to increased mutations [101], changes in gene dosage as a result of copy number changes in the genome [102], or epigenetic changes associated with age [103]. It is also likely that behavioral factors in the fathers that result in delayed marriage also contribute [88], as these factors are very difficult to quantify and correct for in epidemiological studies.

3.4.3 Consequences in Context

From the available data, it is clear that advanced paternal age affects sperm quality, fecundity, and offspring health. However, this topic is only beginning to be thoroughly explored partially due to the recently growing trend of delayed parenthood that

appears to have driven increased media attention toward to the study of advanced paternal age and offspring health. This has placed many physicians in the difficult position of consulting concerned patients regarding their capacity to produce healthy offspring with only scant amounts of data from a field of study in its relative infancy. This discussion is fascinating and extremely complex as a result of the socioeconomic, emotional, and general health issues involved. Physicians should be prepared to address many questions from their patients, but should specifically be able to address two main concerns in this discussion. The first are patients who request to preemptively store sperm at relatively young ages as an alternative to natural conception at an advanced age. The second are male patients who seek advice on the "risks" of having children at advanced age. In either case, the patients must be well informed and comfortable in making their decisions.

Are cryopreserved sperm from a young healthy individual more capable of producing healthy offspring than fresh sperm from the same individual collected at an advanced age? This central question in the paternal aging debate is not easily addressed. In fact, the most accurate answer would be that we simply do not know. It is clear that advanced paternal age has been associated with increased incidence of many disease states in the offspring as has been previously outlined. It is also known that there is a slightly increased risk of birth defects in children conceived through in vitro fertilization (the advanced reproductive technology that would most likely be used in these cases). Additionally, though still controversial, it has been demonstrated that the cryopreservation of sperm, even in the presence of cryomedium, can result in DNA damage thus compounding the problem of using stored samples as an alternative to natural conception at an advanced age [104]. Despite this, because of the low risk in cryopreservation of male gametes, if a patient desires to store sperm at a young age with the intent of future use, it would not be unreasonable to support this decision if the patient has been well educated on the available data.

Patients of advanced age who are considering having children but have not previously stored sperm may also seek medical advice on whether or not they should attempt parenthood based on the recent data that demonstrates increased relative risk to the offspring. Would they be placing their offspring at a significant risk/disadvantage? In response to this question it is important to understand that while the data do suggest a relative increase in the risk of offspring of aged fathers developing many disorders and diseases including, but not limited to schizophrenia, autism and even cancer, the absolute risk of these are still very low. For example, the risk of developing childhood leukemia is approximately 1 in 25,000 in the general public, and in the offspring of older fathers that risk climbs to 1 in 17,000, approximately a 50 % increase [83]. Though the relative risk in this case is statistically significant, the absolute risk to the offspring of an aged father actually developing leukemia remains very low. It will be important for physicians to additionally encourage patients to consider their familial relationships and the emotional benefits of having children and weigh these with the subtle increases in risk of having children at an older age. In consulting male patients of an advanced age, the data do not support the recommendation of halting attempts at conception because of the risks to the offspring as it does in advanced maternal age. Despite this it is important to consider these risks and understand that the cumulative data on the disorders that have relatively increased prevalence in the offspring of older fathers may dissuade some from having children at an older age.

3.5 Conclusions

In recent years, we have learned a great deal regarding the effect of aging on male fertility. Advanced paternal age is negatively associated with many semen parameters, and these negative effects likely drive the general decrease in fertility and fecundity seen in males of advanced age. Though not an abrupt and complete loss of fertility as seen in advanced maternal aging, there is a gradual decrease in gamete quality associated with aging in males. This decrease in quality includes DNA damage, various genetic mutations,

and epigenetic alterations that appear to be capable of causing abnormalities in the offspring. Though we currently have evidence to support the paternal age associated increase in offspring disease susceptibility, the absolute risk remains quite low. Despite this, couples with an aged male partner should consider these risks and discuss them with their health care provider to determine their best course of action in their desire to conceive a child.

References

1. Mills M, Rindfuss RR, McDonald P, et al. Why do people postpone parenthood? Reasons and social policy incentives. Hum Reprod Update. 2011;17(6):848–60.
2. Kuhnert B, Nieschlag E. Reproductive functions of the ageing male. Hum Reprod Update. 2004;10(4):327–39.
3. Bray I, Gunnell D, Davey SG. Advanced paternal age: how old is too old? J Epidemiol Community Health. 2006;60(10):851–3.
4. Australian Bureau of Statistics: Births, Australia, 2011. 2012 [updated October 25, 2012; cited 2013 March 28]; Available from: http://www.abs.gov.au/ausstats/abs@.nsf/mf/3301.0.
5. Kidd SA, Eskenazi B, Wyrobek AJ. Effects of male age on semen quality and fertility: a review of the literature. Fertil Steril. 2001;75(2):237–48.
6. Eskenazi B, Wyrobek AJ, Sloter E, et al. The association of age and semen quality in healthy men. Hum Reprod. 2003;18(2):447–54.
7. Sartorius GA, Nieschlag E. Paternal age and reproduction. Hum Reprod Update. 2010;16(1):65–79.
8. Kuhnert B, Nieschlag E. Reproductive functions of the ageing male. Hum Reprod Update. 2004;10(4):327–39.
9. Hellstrom WJ, Overstreet JW, Sikka SC, et al. Semen and sperm reference ranges for men 45 years of age and older. J Androl. 2006;27(3):421–8.
10. Johnson FB, Sinclair DA, Guarente L. Molecular biology of aging. Cell. 1999;96(2):291–302.
11. Guarente L, Kenyon C. Genetic pathways that regulate ageing in model organisms. Nature. 2000;408(6809):255–62.
12. Humm KC, Sakkas D. Role of increased male age in IVF and egg donation: is sperm DNA fragmentation responsible? Fertil Steril. 2013;99(1):30–6.
13. Schmid TE, Eskenazi B, Baumgartner A, et al. The effects of male age on sperm DNA damage in healthy non-smokers. Hum Reprod. 2007;22(1):180–7.
14. Wyrobek AJ, Eskenazi B, Young S, et al. Advancing age has differential effects on DNA damage, chromatin integrity, gene mutations, and aneuploidies in sperm. Proc Natl Acad Sci USA. 2006;103(25):9601–6.
15. Varshini J, Srinag BS, Kalthur G, et al. Poor sperm quality and advancing age are associated with increased sperm DNA damage in infertile men. Andrologia. 2012;44 Suppl 1:642–9.
16. Moskovtsev SI, Willis J, Mullen JB. Age-related decline in sperm deoxyribonucleic acid integrity in patients evaluated for male infertility. Fertil Steril. 2006;85(2):496–9.
17. Cocuzza M, Athayde KS, Agarwal A, et al. Age-related increase of reactive oxygen species in neat semen in healthy fertile men. Urology. 2008;71(3):490–4.
18. Colin A, Barroso G, Gomez-Lopez N, et al. The effect of age on the expression of apoptosis biomarkers in human spermatozoa. Fertil Steril. 2010;94(7):2609–14.
19. Jones KT. Meiosis in oocytes: predisposition to aneuploidy and its increased incidence with age. Hum Reprod Update. 2008;14(2):143–58.
20. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet. 2001;2(4):280–91.
21. Handyside AH. Molecular origin of female meiotic aneuploidies. Biochim Biophys Acta. 2012;1822(12):1913–20.
22. Luetjens CM, Rolf C, Gassner P, et al. Sperm aneuploidy rates in younger and older men. Hum Reprod. 2002;17(7):1826–32.
23. Bosch M, Rajmil O, Martinez-Pasarell O, et al. Linear increase of diploidy in human sperm with age: a four-colour FISH study. Eur J Hum Genet. 2001;9(7):533–8.
24. Griffin DK, Abruzzo MA, Millie EA, et al. Non-disjunction in human sperm: evidence for an effect of increasing paternal age. Hum Mol Genet. 1995;4(12):2227–32.
25. Lowe X, Eskenazi B, Nelson DO, et al. Frequency of XY sperm increases with age in fathers of boys with Klinefelter syndrome. Am J Hum Genet. 2001;69(5):1046–54.
26. Conrad DF, Keebler JE, DePristo MA, et al. Variation in genome-wide mutation rates within and between human families. Nat Genet. 2011;43(7):712–4.
27. Wang J, Fan HC, Behr B, et al. Genome-wide single-cell analysis of recombination activity and de novo mutation rates in human sperm. Cell. 2012;150(2):402–12.
28. Lynch M. Rate, molecular spectrum, and consequences of human mutation. Proc Natl Acad Sci USA. 2010;107(3):961–8.
29. Crow JF. The origins, patterns and implications of human spontaneous mutation. Nat Rev Genet. 2000;1(1):40–7.
30. Penrose LS. Parental age and mutation. Lancet. 1955;269(6885):312–3.
31. Kong A, Frigge ML, Masson G, et al. Rate of de novo mutations and the importance of father's age to disease risk. Nature. 2012;488(7412):471–5.
32. Ferreira MG, Miller KM, Cooper JP. Indecent exposure: when telomeres become uncapped. Mol Cell. 2004;13(1):7–18.
33. Allsopp RC, Vaziri H, Patterson C, et al. Telomere length predicts replicative capacity of human

fibroblasts. Proc Natl Acad Sci USA. 1992;89(21): 10114–8.
34. Unryn BM, Cook LS, Riabowol KT. Paternal age is positively linked to telomere length of children. Aging Cell. 2005;4(2):97–101.
35. Njajou OT, Cawthon RM, Damcott CM, et al. Telomere length is paternally inherited and is associated with parental lifespan. Proc Natl Acad Sci USA. 2007;104(29):12135–9.
36. Aviv A. Genetics of leukocyte telomere length and its role in atherosclerosis. Mutat Res. 2012;730(1–2): 68–74.
37. Smith RG, Kember RL, Mill J, et al. Advancing paternal age is associated with deficits in social and exploratory behaviors in the offspring: a mouse model. PLoS One. 2009;4(12):e8456.
38. Naserbakht M, Ahmadkhaniha HR, Mokri B, et al. Advanced paternal age is a risk factor for schizophrenia in Iranians. Ann Gen Psychiatry. 2011;10:15.
39. Dalman C. Advanced paternal age increases risk of bipolar disorder in offspring. Evid Based Ment Health. 2009;12(2):59.
40. Wiener-Megnazi Z, Auslender R, Dirnfeld M. Advanced paternal age and reproductive outcome. Asian J Androl. 2012;14(1):69–76.
41. Alio AP, Salihu HM, McIntosh C, et al. The effect of paternal age on fetal birth outcomes. Am J Mens Health. 2012;6(5):427–35.
42. Eriksen W, Sundet JM, Tambs K. Paternal age at birth and the risk of obesity in young adulthood: a register-based birth cohort study of norwegian males. Am J Hum Biol. 2013;25(1):29–34.
43. Wilson VL, Jones PA. DNA methylation decreases in aging but not in immortal cells. Science. 1983;220(4601):1055–7.
44. Oakes CC, Smiraglia DJ, Plass C, et al. Aging results in hypermethylation of ribosomal DNA in sperm and liver of male rats. Proc Natl Acad Sci USA. 2003;100(4):1775–80.
45. Thompson RF, Atzmon G, Gheorghe C, et al. Tissue-specific dysregulation of DNA methylation in aging. Aging Cell. 2010;9(4):506–18.
46. Adkins RM, Thomas F, Tylavsky FA, et al. Parental ages and levels of DNA methylation in the newborn are correlated. BMC Med Genet. 2011;12:47.
47. Tominaga K, Pereira-Smith OM. The role of chromatin reorganization in the process of cellular senescence. Curr Drug Targets. 2012;13(13):1593–602.
48. Nijs M, De Jonge C, Cox A, et al. Correlation between male age, WHO sperm parameters, DNA fragmentation, chromatin packaging and outcome in assisted reproduction technology. Andrologia. 2011;43(3):174–9.
49. Hassan MA, Killick SR. Effect of male age on fertility: evidence for the decline in male fertility with increasing age. Fertil Steril. 2003;79 Suppl 3:1520–7.
50. de la Rochebrochard E, Thonneau P. Paternal age and maternal age are risk factors for miscarriage; results of a multicentre European study. Hum Reprod. 2002;17(6):1649–56.
51. Selvin S, Garfinkel J. Paternal age, maternal age and birth order and the risk of a fetal loss. Hum Biol. 1976;48(1):223–30.
52. Ford WC, North K, Taylor H, et al. Increasing paternal age is associated with delayed conception in a large population of fertile couples: evidence for declining fecundity in older men. The ALSPAC Study Team (Avon Longitudinal Study of Pregnancy and Childhood). Hum Reprod. 2000;15(8):1703–8.
53. Dunson DB, Colombo B, Baird DD. Changes with age in the level and duration of fertility in the menstrual cycle. Hum Reprod. 2002;17(5):1399–403.
54. Olsen J. Subfecundity according to the age of the mother and the father. Dan Med Bull. 1990;37(3): 281–2.
55. Belloc S, Cohen-Bacrie P, Benkhalifa M, et al. Effect of maternal and paternal age on pregnancy and miscarriage rates after intrauterine insemination. Reprod Biomed Online. 2008;17(3):392–7.
56. Mathieu C, Ecochard R, Bied V, et al. Cumulative conception rate following intrauterine artificial insemination with husband's spermatozoa: influence of husband's age. Hum Reprod. 1995;10(5):1090–7.
57. Bellver J, Garrido N, Remohi J, et al. Influence of paternal age on assisted reproduction outcome. Reprod Biomed Online. 2008;17(5):595–604.
58. Klonoff-Cohen HS, Natarajan L. The effect of advancing paternal age on pregnancy and live birth rates in couples undergoing in vitro fertilization or gamete intrafallopian transfer. Am J Obstet Gynecol. 2004;191(2):507–14.
59. de La Rochebrochard E, de Mouzon J, Thepot F, et al. Fathers over 40 and increased failure to conceive: the lessons of in vitro fertilization in France. Fertil Steril. 2006;85(5):1420–4.
60. Frattarelli JL, Miller KA, Miller BT, et al. Male age negatively impacts embryo development and reproductive outcome in donor oocyte assisted reproductive technology cycles. Fertil Steril. 2008;90(1): 97–103.
61. Whitcomb BW, Turzanski-Fortner R, Richter KS, et al. Contribution of male age to outcomes in assisted reproductive technologies. Fertil Steril. 2011;95(1): 147–51.
62. Risch N, Reich EW, Wishnick MM, et al. Spontaneous mutation and parental age in humans. Am J Hum Genet. 1987;41(2):218–48.
63. Lim J, Maher GJ, Turner GD, et al. Selfish spermatogonial selection: evidence from an immunohistochemical screen in testes of elderly men. PLoS One. 2012;7(8):e42382.
64. Moloney DM, Slaney SF, Oldridge M, et al. Exclusive paternal origin of new mutations in Apert syndrome. Nat Genet. 1996;13(1):48–53.
65. Wilkin DJ, Szabo JK, Cameron R, et al. Mutations in fibroblast growth-factor receptor 3 in sporadic cases of achondroplasia occur exclusively on the paternally derived chromosome. Am J Hum Genet. 1998; 63(3):711–6.

66. Sol-Church K, Stabley DL, Nicholson L, et al. Paternal bias in parental origin of HRAS mutations in Costello syndrome. Hum Mutat. 2006;27(8):736–41.
67. Tartaglia M, Cordeddu V, Chang H, et al. Paternal germline origin and sex-ratio distortion in transmission of PTPN11 mutations in Noonan syndrome. Am J Hum Genet. 2004;75(3):492–7.
68. Carlson KM, Bracamontes J, Jackson CE, et al. Parent-of-origin effects in multiple endocrine neoplasia type 2B. Am J Hum Genet. 1994;55(6): 1076–82.
69. Goriely A, Wilkie AO. Paternal age effect mutations and selfish spermatogonial selection: causes and consequences for human disease. Am J Hum Genet. 2012;90(2):175–200.
70. MacDonald ME, Ambrose CM, Duyao MP, et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell. 1993;72(6):971–83.
71. Duyao M, Ambrose C, Myers R, et al. Trinucleotide repeat length instability and age of onset in Huntington's disease. Nat Genet. 1993;4(4):387–92.
72. Goldberg YP, Kremer B, Andrew SE, et al. Molecular analysis of new mutations for Huntington's disease: intermediate alleles and sex of origin effects. Nat Genet. 1993;5(2):174–9.
73. Brunner HG, Bruggenwirth HT, Nillesen W, et al. Influence of sex of the transmitting parent as well as of parental allele size on the CTG expansion in myotonic dystrophy (DM). Am J Hum Genet. 1993; 53(5):1016–23.
74. Zheng CJ, Byers B, Moolgavkar SH. Allelic instability in mitosis: a unified model for dominant disorders. Proc Natl Acad Sci USA. 1993;90(21):10178–82.
75. Fisch H, Hyun G, Golden R, et al. The influence of paternal age on down syndrome. J Urol. 2003;169(6): 2275–8.
76. Zaragoza MV, Jacobs PA, James RS, et al. Nondisjunction of human acrocentric chromosomes: studies of 432 trisomic fetuses and liveborns. Hum Genet. 1994;94(4):411–7.
77. Sloter E, Nath J, Eskenazi B, et al. Effects of male age on the frequencies of germinal and heritable chromosomal abnormalities in humans and rodents. Fertil Steril. 2004;81(4):925–43.
78. Fonseka KG, Griffin DK. Is there a paternal age effect for aneuploidy? Cytogenet Genome Res. 2011; 133(2–4):280–91.
79. De Souza E, Morris JK. Case–control analysis of paternal age and trisomic anomalies. Arch Dis Child. 2010;95(11):893–7.
80. Zhu JL, Madsen KM, Vestergaard M, et al. Paternal age and congenital malformations. Hum Reprod. 2005;20(11):3173–7.
81. De Souza E, Alberman E, Morris JK. Down syndrome and paternal age, a new analysis of case–control data collected in the 1960s. Am J Med Genet A. 2009;149A(6):1205–8.
82. Oksuzyan S, Crespi CM, Cockburn M, et al. Birth weight and other perinatal characteristics and childhood leukemia in California. Cancer Epidemiol. 2012;36(6):e359–65.
83. Murray L, McCarron P, Bailie K, et al. Association of early life factors and acute lymphoblastic leukaemia in childhood: historical cohort study. Br J Cancer. 2002;86(3):356–61.
84. Hemminki K, Kyyronen P, Vaittinen P. Parental age as a risk factor of childhood leukemia and brain cancer in offspring. Epidemiology. 1999;10(3):271–5.
85. Yip BH, Pawitan Y, Czene K. Parental age and risk of childhood cancers: a population-based cohort study from Sweden. Int J Epidemiol. 2006;35(6):1495–503.
86. Choi JY, Lee KM, Park SK, et al. Association of paternal age at birth and the risk of breast cancer in offspring: a case control study. BMC Cancer. 2005;5:143.
87. Zhang Y, Kreger BE, Dorgan JF, et al. Parental age at child's birth and son's risk of prostate cancer. The Framingham Study. Am J Epidemiol. 1999;150(11): 1208–12.
88. Hare EH, Moran PA. Raised parental age in psychiatric patients: evidence for the constitutional hypothesis. Br J Psychiatry. 1979;134:169–77.
89. Miller B, Messias E, Miettunen J, et al. Meta-analysis of paternal age and schizophrenia risk in male versus female offspring. Schizophr Bull. 2011;37(5):1039–47.
90. Matheson SL, Shepherd AM, Laurens KR, et al. A systematic meta-review grading the evidence for non-genetic risk factors and putative antecedents of schizophrenia. Schizophr Res. 2011;133(1–3):133–42.
91. Wohl M, Gorwood P. Paternal ages below or above 35 years old are associated with a different risk of schizophrenia in the offspring. Eur Psychiatry. 2007;22(1): 22–6.
92. Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. Br J Psychiatry. 2009;195(1):7–14.
93. Hultman CM, Sandin S, Levine SZ, et al. Advancing paternal age and risk of autism: new evidence from a population-based study and a meta-analysis of epidemiological studies. Mol Psychiatry. 2011;16(12): 1203–12.
94. Frans EM, Sandin S, Reichenberg A, et al. Advancing paternal age and bipolar disorder. Arch Gen Psychiatry. 2008;65(9):1034–40.
95. Menezes PR, Lewis G, Rasmussen F, et al. Paternal and maternal ages at conception and risk of bipolar affective disorder in their offspring. Psychol Med. 2010;40(3):477–85.
96. Kuja-Halkola R, Pawitan Y, D'Onofrio BM, et al. Advancing paternal age and offspring violent offending: a sibling-comparison study. Dev Psychopathol. 2012;24(3):739–53.
97. Saha S, Barnett AG, Buka SL, et al. Maternal age and paternal age are associated with distinct childhood behavioural outcomes in a general population birth cohort. Schizophr Res. 2009;115(2–3):130–5.
98. Malaspina D, Reichenberg A, Weiser M, et al. Paternal age and intelligence: implications for age-related genomic changes in male germ cells. Psychiatr Genet. 2005;15(2):117–25.

99. Saha S, Barnett AG, Foldi C, et al. Advanced paternal age is associated with impaired neurocognitive outcomes during infancy and childhood. PLoS Med. 2009;6(3):e40.
100. Svensson AC, Abel K, Dalman C, et al. Implications of advancing paternal age: does it affect offspring school performance? PLoS One. 2011;6(9): e24771.
101. Reichenberg A, Gross R, Weiser M, et al. Advancing paternal age and autism. Arch Gen Psychiatry. 2006;63(9):1026–32.
102. Hehir-Kwa JY, Rodriguez-Santiago B, Vissers LE, et al. De novo copy number variants associated with intellectual disability have a paternal origin and age bias. J Med Genet. 2011;48(11):776–8.
103. Perrin MC, Brown AS, Malaspina D. Aberrant epigenetic regulation could explain the relationship of paternal age to schizophrenia. Schizophr Bull. 2007;33(6):1270–3.
104. Zribi N, Feki Chakroun N, El Euch H, et al. Effects of cryopreservation on human sperm deoxyribonucleic acid integrity. Fertil Steril. 2010;93(1):159–66.

Testosterone Replacement Therapy in Men: Effects on Fertility and Health

4

Peter T.K. Chan

4.1 Introduction

A rising volume of the current literature has demonstrated the safety and health benefits of testosterone replacement therapy for late-onset hypogonadism in men. The simultaneous increase in the coverage both by the lay media and the internet has allowed public awareness of the notion of "andropause" to growth tremendously. As a result of this growing demand from patients for evaluation, counseling and treatment of late-onset hypogonadism along with the increased knowledge, comfort levels, and willingness of healthcare professionals in managing the condition [1], the number of prescriptions of testosterone replacement therapy (TRT) has exploded in the past decade. The levels of sales of TRT products were estimated to have increased by 500 % from 1993 [2]. In a comprehensive global report on TRT market, Global Industry Analysts projected the global TRT market to reach $5.0 billion in 2017. In response to such a paradigm shift of late-onset hypogonadism management resulting in a rapidly growing consumer market, the pharmaceutical products available for testosterone replacement therapy not only have increased in their varieties but, more importantly, have also undergone significant modifications on various aspects such as the improvement of safety, bioavailability, and cost-effectiveness. A growing volume of the adult male population is expected to be on testosterone replacement, possibly as a life-long therapy for hypogonadism. The focus of this chapter is on the use of testosterone replacement therapy for late-onset hypogonadism in men and its potential impact on the general and reproductive health.

4.2 Hypogonadism in Adult Male

Clinically, male hypogonadism refers to the state of health where there is a deficiency of androgen activity. Male hypogonadism may be due to intrinsic testicular failure in testosterone production and spermatogenesis, a condition commonly referred to as primary hypogonadism. On the other hand, when hypogonadism is caused by inadequate gonadal stimulation from the hypothalamus–pituitary axis production and release of gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH), it is referred to as secondary (or central) hypogonadism. Other medical conditions such as hemochromatosis, diabetes, severe malnutrition, and febrile illnesses may also interfere with normal gonadal function leading to hypogonadism [3].

P.T.K. Chan, M.D., C.M., M.Sc., F.R.C.S.(C)., F.A.C.S. (✉)
Male Reproductive Medicine, S 6.95, Department of Urology, McGill University Health Center, 687 Pine Avenue West, Montreal, QC, Canada H3A 1A1
e-mail: mcgillurology@yahoo.com

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_4,

Table 4.1 Presentations of late-onset hypogonadism in men

Erectile dysfunction
Decreased sexual desire
Mood changes
Cognition/memory impairment
Lack of energy/motivation
Sleep disturbances
Quality of life
Anemia
Dyslipidemia
Sarcopenia
Loss of body hair (axillary and pubic)
Loss of height
Osteopenia/osteoporosis
Low-impact fracture
Loss of muscle mass
Decrease in physical strength and performance
Hot flushes
Testicular hypotrophy
Central obesity

Of note, secondary hypogonadism may be congenital and iatrogenic. Idiopathic hypogonadotropic hypogonadism (IHH) with normal sense of smell (normosmic) or with anosmia (Kallmann syndrome) is a rare genetic disorder caused by an isolated defect in the secretion of GnRH (gonadotropin-releasing hormone) by the hypothalamus, or, less frequently, by a defect in the action of GnRH on pituitary gonadoropes [4]. Iatrogenic causes of secondary hypogonadism include surgical removal of the pituitary gland for treatment of tumors such as craniopharyngioma and pituitary adenoma. Traumatic damage to the pituitary gland is another cause of secondary hypogonadism. In these cases where the direct cause of hypogonadism is specifically secondary to gonadotropins deficiency, replacement therapy with gonadotropins or GnRH (for IHH) is an established effective therapy to resume gonadal function for both spermatogeneis and testosterone production.

Our focus of discussion, however, will be on late-onset hypogonadism (LOH) for which the common choice of pharmacological management is replacement therapy with testosterone or testosterone-based products. LOH refers specifically to a cluster of presentations (Table 4.1) that appeared in adulthood secondary to a decline in androgen activity. Various studies have indicated a gradual decline in serum testosterone levels in men with increasing age. In the media, this condition is often referred to with the laymen's terms "andropause" or "man-opause" (as an anology menopause in female). Other more precise terms used to describe this syndrome include testosterone deficiency syndrome (TDS) and partial androgen deficiency in aging men (PADAM).

4.2.1 Insulin Resistance

Serum testosterone levels decline 1–1.5 % per year after age 30 years [5]. At the level of the hypothalamus, pulsatile release of GnRH is thought to be reduced in quantity with old age with possible loss of the circadian rhythm. At the testicular level, Leydig cell response to GnRH is also blunted with aging. This, combined with increase of the levels of sex hormone binding globulin, results in decrease in the free level of testosterone. The true prevalence of hypogonadism varies in different reports depending on the age group, health status, ethnicity, and other factors [6]. The Massachusetts Male Aging Study demonstrated that the prevalence of hypogonadism in men ranges from 6.0 to 12.3 % between the ages of 40 and 69 years and estimated that 2.4 million men in the USA have androgen deficiency [7]. A more recent report estimated the prevalence of low testosterone (serum total testosterone < 300 ng/dL or 10.4 nmol/L) to be as high as 38.7 % in males over the age of 45 in outpatient primary care populations [8].

Hypogonadism has also been linked to general health conditions such as dyslipidemias [9], type II diabetes [10–12], metabolic syndrome [13], and even increased mortality. Shores et al. [14] followed over 800 male veterans for an average of 4.3 years and found that low serum testosterone was associated with higher all-cause mortality. Similar conclusions was drawn by Khaw et al. [15] who followed over 11,000 men aged 40–70 year for 10 years and reported the associated risks of low endogenous testosterone

with elevated risks of all-cause mortality, cardiovascular-related mortality, and cancer-related mortality. Men in the highest quartile testosterone levels were found to have 30 % reduction in mortality compared with those in the lowest quartile. In an epidemiologic model developed to quantify the impact of hypogonadism (using a prevalence of 13.4 %) as a predisposing factor for men's health, Moskovic et al. [16] determined that, over a 20-year period, hypogonadism is projected to be involved in the development of approximately 1.3 million new cases of cardiovascular disease, 1.1 million new cases of diabetes mellitus, and over 600,000 of osteoporosis-related fracture, with an attributed cost burden of these diseases estimated to be $190–$525 billion in inflation-adjusted healthcare expenditures in the USA.

4.3 Benefits of Testosterone Replacement Therapy on General Health

Since the first published case in Lancet in 1889 by Dr. Charles Brown-Sequard on self-injection of testicular extracts from animals resulting in increased energy, muscle strength, stamina, and mental agility [17], a wealth of literature, particularly from the past three decades, has reported various general health benefits of various forms of testosterone replacement therapy (TRT) on men with hypogonadism. Controversies do exist on whether TRT is efficacious in providing benefits to men with late-onset hypogonadism on various health issues. Since most of the current interventional studies are short term and non-placebo controlled with heterogenous baseline parameters and different designs in the outcome measured and analyzed, it is challenging to delineate what subgroups of patients will have the maximal benefits of TRT to have long-lasting improvement on the various aspects of their general health. Ideally, large-scale, multicentered, long-termed randomized, placebo-controlled trials are needed to fully establish not only the long-termed efficacy but also the potential health risks of TRT. A new multicentered clinical trial, sponsored by The National Institute on Aging of the National Institutes of Health of the United States, is expected to complete by mid-2015 (http://www.clinicaltrials.gov/ct2/show/NCT00799617?term=testosterone+aging&rank=40) and should provide more definitive answers to potential benefits of TRT in aging men. However, it is not powered to assess all potential risks such as prostate cancer and cardiovascular events [18]. Thus, clinicians should be cautious in drawing their conclusions using the evidence-based results available from the current literature. Various clinical recommendations and guidelines have recently been published by reputable societies of interest on the evaluation, counseling, management, and monitoring for men with late-onset hypogonadism [19–21].

4.3.1 Fat and Muscle Composition

Increase in lean muscle mass, particularly in the trunk, along with decrease in fat mass in the extremities, have been reported with TRT in elderly men [22, 23]. The translation of these positive effects of TRT on muscle strength, motor performance, and fall prevention, however, is controversial [24]. In men with significant comorbidity such as chronic obstructive pulmonary disease [25], men receiving glucocorticoids [26], and frail and elderly men in rehabilitation [23, 27–29], improvement in muscle strength or physical function after TRT has been reported. In healthy elderly men, on the other hand, three randomized, placebo-controlled trials with 6 [30] and 36 months [22] of treatment failed to demonstrate improvement in muscle strength.

4.3.2 Bone Composition

Hypogonadism is a known cause of osteoporosis and osteopenia. Rapid bone loss is observed after castration and androgen deprivation therapy [31]. Bone microarchitecture and cortical and trabecular bone mineral density are impaired in men with hypogonadism [32], resulting in increased risks of bone fractures [33, 34]. The prevalence of

hypogonadism was found to be ~60–70 % in men with hip fractures [35–37] and up to 20 % in men with vertebral fracture [38]. Several interventional studies, including placebo-controlled studies and meta-analyses, reported increase in bone mineral density after TRT for hypogonadism, with greater increase in the lumbar spine than in the hip [24, 39–43]. However, there is currently insufficient data to determine the efficacy of TRT on reducing the risk of bone fracture.

4.3.3 Sexual Function

Reduced libido or sex drive has been associated with hypogonadism [44, 45]. The association of erectile function with serum testosterone levels, on the other hand, is less clear [45–47]. It appears that when it is clearly subnormal (<320 ng/dL or 11 nmol/L), there is a syndromic association with decreased serum total testosterone levels with sexual symptoms such as morning erection, low sexual desire, and erectile function [48]. A recent 6-month randomized controlled trial on TRT in men with testosterone level <395 ng/dL or 13.7 nmol/L failed to demonstrate a benefit on sexual functioning [30]. However, three meta-analyses of published studies including randomized placebo-controlled trials [48–51] revealed improvement on male sexual function with testosterone replacement therapy. The meta-regression analysis [51] demonstrated that the effect of TRT on erectile function was inversely related to the baseline testosterone concentration. Hence, the more severe the hypogonadism, the more significant or impressive are the results obtained with TRT. Minimal or no effect was observed for baseline testosterone levels above 345 ng/dL or 12 nmol/L. Age appears to be another important moderator in evaluating the effect of TRT on sexual function. Boloña [50] reported a sizable and significant effect of TRT on erectile function in trials including young patients and a minimal and nonsignificant effect in those including older ones (mean age > 50 years). One presumable explanation for this observation is that hypogonadism in younger patients may be a main cause of sexual dysfunction while for older men it may be one element of a multifactorial sexual dysfunction. The beneficial effects of TRT on sexual function are also seen in studies on the combined used of testosterone and phostphodiesterase-5 inhibitors (PDE5I's) for erectile dysfunction. These studies [52–56] demonstrated that the addition of TRT can salvaged 37.5–92 % of subjects who failed to respond to PDE5I's alone.

4.3.4 Mood and Quality of Life

Hypogonadism is associated with depressive symptoms, impaired cognitive function, and symptoms of dementia [19, 57–59], though such an association is weak. In a recent systemic meta-analysis evaluated seven placebo-controlled, randomized trials ($n=364$) comparing testosterone replacement with placebo in depressed men, Zarrouf et al. [60] reported a significant positive response to TRT in hypogonadal patients. TRT is beneficial on mood only in men with clear subnormal testosterone levels [61]. But for hypogonadal with severe depression, the benefits of TRT on depressive symptoms seem less significant. In a recent placebo-controlled trial, Pope et al. [62] failed to show any benefit of TRT in depressed hypogonadal men (serum total testosterone<350 ng/dL or 12.1 nmol/mL) who were resistant to selective serotonin reuptake inhibitor as a standard antidepressant treatment.

The results of randomized controlled trials on the effects of TRT on quality of life, as assessed by various questionnaires, yield mixed results. In a 6-month TRT trial with 1 % testosterone gel followed by 12 months of open-label follow-up, Behre et al. [63] reported a significant benefit on the health-related quality of life in the TRT group over the controlled group, particularly in the psychological and sexual subscale scores. In another trial from China using 6 months of oral testosterone undecanoate, quality of life measured by the Short Form Health Survey-12 significantly improved in the TRT group [64]. Similar findings were confirmed by in a 12-month trial with intramuscular testosterone undecanoate in Malaysian subjects [65, 66]. Other trials [27, 30] failed to demonstrate a significant improvement in the quality of life of hypogonadal men treated with TRT.

4.3.5 Components of Metabolic Syndrome

Metabolic syndrome, previously also known as syndrome X, has several components as described by the International Diabetes Federation in a consensus worldwide definition in 2006 [67]. These components include: increased triglyceride levels (>150 mg/dL or 1.7 mmol/L), reduced high-density lipid (HDL) levels (<40 mg/dL or 1.03 mmol/L in males), elevated blood pressure (systolic > 130 or diastolic > 85 mmHg), glucose intolerance (fasting plasma glucose > 100 mg/dL or 5.6 mmol/L), and central obesity. Hypogonadism is common in men with type II diabetes or metabolic syndrome. Men with hypogonadism seem to have an increased risk of subsequent development of type II diabetes and metabolic syndrome. Various studies have reported an inverse relationship between testosterone levels and insulin resistance, dyslipidemia, and central obesity [68, 69]. However, it is uncertain if hypogonadism is a cause or a consequence of metabolic syndrome. Studies have reported that visceral obesity can be a potential cause of hypogonadism but hypogonadism may well be a cause of obesity and insulin resistance [69]. The association of these various components of metabolic syndrome clearly establishes a vicious cycle leading to disease progression.

Several interventional studies demonstrated the beneficial effects of TRT on various metabolic parameters including blood pressure, insulin resistance, lipid profile, body composition, and glycosylated hemoglobin (HbA1c) levels. Isidori et al. [42] reported that TRT in middle-aged men leads to reduction in fat mass and total cholesterol. In a meta-analysis, Whitsel et al. [70] showed a dose-dependent decrease in total cholesterol, low- and high-density lipoprotein-cholesterol. In patients with type II diabetes, TRT was associated with a significant reduction of fasting plasma glucose, HbA1c, fat mass, and triglycerides [71]. For patients with established metabolic syndrome, TRT appears to significantly reduce fasting plasma glucose, Homeostatic Model Assessment (HOMA) index, triglycerides and waist circumference, as well as with an increase of HDL-Cholesterol [69].

4.3.6 Cognitive Function

Barrett-Connor et al. [57] reported high endogenous testosterone, and low estradiol levels predicted improved performance on cognitive function. In short-term interventional studies with TRT, Cherrier et al. [72–74] demonstrated improvements in verbal and spatial memory in healthy men and also in men with Alzheimer disease or mild cognitive impairment. Conflicting results, however, were reported by longer trials [30, 75].

4.3.7 Cardiovascular Function

Current studies by various investigators suggested a link between hypogonadism and increased risks of cardiovascular diseases [76–80]. However, it remains uncertain if low T plays a direct pathogenic role in increasing cardiovascular risks. Hypogonadism may well be a marker of preexisting cardiovascular disease rather than an independent risk factor. The suppressing effects of various chronic diseases including metabolic syndrome and type II diabetes on testosterone levels lead Corona et al. [81] to hypothesize that low T during chronic diseases represents a protective or adaptive mechanism to turn off testosterone-dependent function such as reproduction and physical labor that are less desired when the general physical condition is ailing.

With regard to the effects of TRT on cardiovascular risks, a recent double-blinded placebo-controlled study on men with metabolic syndrome showed that TRT may delay the progression of atherosclerosis, as detected by carotid intima media thickness, and the level of high-sensitivity C-reactive protein [82]. Three meta-analyses [83–85] found no significant benefit of TRT for cardiovascular events. However, the statistical power of these analyses is significantly limited by the small sample series and short duration of study duration. In recent reviews [86, 87], there are over a dozen of recent studies that demonstrated the beneficial effects of TRT on angina with positive effects such as decrease frequency of angina, increase in exercise tolerance, and time

to ischemia. A recent randomized controlled trial [27] of TRT on frail elderly men at the maximum recommended dose of TRT (with 10 mg per day of 1 % testosterone gel) reported a high rate of TRT-associated CV adverse events. This trial, however, was criticized by Morgentaler [88] that: (1) there was no rigorous cardiovascular assessment in the trial where nearly half of the cardiovascular events were self-reported or obtained from outside medical sources; (2) the TRT group had more cardiovascular risk factors at baseline than the placebo group; (3) the cardiovascular events consisted of a wide variety of symptoms and findings that are not specific for cardiovascular diseases such as peripheral edema and syncope. Additional studies are thus required to further evaluate if TRT can truly benefit hypogonadal men in reducing not only cardiovascular risks but also the event-specific mortality rate.

4.4 Side Effects of TRT

4.4.1 Prostate Health

The most significant concern amongst all TRT adverse events is on prostate health. Prostate tissues are androgen responsive. In a case series, Favilla et al. [89] reported that age and total serum testosterone correlate with LUTS as measured by International Prostate Symptom Score (IPSS). But after adjusting for various confounding factors, other studies [90–92] failed to confirm an association between higher serum testosterone levels with worse lower urinary tract symptoms (LUTS). On the contrary, more recent studies demonstrated an inverse relationship between total testosterone, DHT, and the development of LUTS [93, 94]. With regard to TRT, an early meta-analysis in 2005 of randomized, placebo-controlled studies on TRT [84] showed a higher risk in the TRT groups of detection of all prostate events, defined as incidence of prostate cancer, increase in IPSS, increase in prostate-specific antigen (PSA), and acute urinary retention. Subsequently, however, a number of short-term (<1 year) studies demonstrated little negative effect on urinary function or prostate volume (reviewed by Shigehara & Namiki [95]). In fact, several studies, including one randomized controlled trial, demonstrated that TRT may actually improve LUTS [96–102]. It should, however, be kept in mind that most of these studies focused on men with mild to moderate degree of LUTS. For men with severe LUTS (e.g., with high IPSS score above 19 points), TRT remains contraindicated as there exists a risks of increase in prostate volume [99] that may theoretically increase the risks of urinary retention. Further studies including long-term observations and many patients with a wide range of severities of LUTS are required to reach more definitive conclusions of TRT on LUTS.

Prostate cancer represents one of the most commonly diagnosed cancers in men over the age of 40 years. Like normal prostate tissues, prostate adenocarcinoma is also androgen responsive. With the initial report by Huggins in 1941 [103] on androgen ablation therapy causing regression of metastatic prostate cancer, a work for which he was awarded the Nobel Prize for Physiology and Medicine in 1966, it was once thought that TRT would lead to development and progression of prostate cancer. However, an extensive review of the current literature, including several large longitudinal studies of up to 20 years of duration, with over 400,000 men studied, failed to establish a direct link between prostate cancer and high testosterone levels [104]. The most recent placebo-controlled randomized trial of TRT revealed no increase in prostate volume, no change in biomarkers of cell proliferation and angiogenesis, and no increase in prostate cancer cases [105]. A longer trial of TRT for 3 years showed no significant changes in PSA levels beyond 6 months of treatment [22]. A recent trial of over 6 years of TRT showed no relevant changes in PSA concentration, PSA velocity, or any significant prostate cancer risks [106].

For men received TRT with localized prostate cancer treated with radical prostatectomy [107–109], radiation therapy [110, 111], or brachytherapy [112], the risk of biochemical recurrence, as indicated by a significant increase in serum PSA level, was estimated to be 2 of 111 men (1.8 %) [113], not as high as one would

expect should TRT really increase the risks of prostate cancer recurrence. Even for men with untreated low grade localized prostate cancer (Gleason score 6 or 7 out of 10 at initial biopsy), TRT for a median of 2.5 years (range 1.0–8.1 years) was not associated with prostate cancer progression. As Morgentaler [113] stated, although there are as yet no large-scale, long-term controlled studies of T therapy to provide a definitive assessment of risk, numerous smaller clinical trials as well as population-based longitudinal studies consistently failed to support the historical idea that T therapy poses an increased risk of prostate cancer or exacerbation of symptoms due to benign prostatic hyperplasia.

Currently manufacturers for all products for TRT have included statements in product inserts that TRT is contraindicated for men with or suspected prostate cancer. Indeed for men with advanced or metastatic prostate cancer that require androgen ablation, TRT should remain an absolute contraindication (consistent with the conclusion of the original report by Huggins in 1941 [103]). Likewise for men with prostate cancer demonstrating factors of high risk of biochemical recurrence (such as extraprostatic extension, positive margins, or lymph nodes at surgery, Gleason scores of 8 or more on biopsy and invasion of the seminal vesicles), clinician must exercise caution when considering the use of TRT.

4.4.2 Polycythemia

Polycythemia, as indicated by an elevation of hematocrit above 50 %, is the most frequent TRT-related adverse event in most clinical trials. In a meta-analysis of 19 randomized controlled trials with 651 subjects on TRT and 433 on placebo [85], TRT increased the risk of polycythemia over placebo by four times. In a more recent meta-analysis of adverse events, Fernández-Balsells et al. [83] reported that TRT was associated with a significant increase in hematocrit (3.18 %; 95 % CI 1.35–5.01), hemoglobin (0.80 g/dL; 95 % CI 0.45–1.14), and a decrease in high-density lipoprotein (HDL) cholesterol (–0.49 mg/dL; 95 % CI –0.85 to 0.13). Thus, careful monitoring of this parameter to allow dosage reduction or treatment discontinuation is important for all men on TRT.

4.4.3 Gynecomastia

Gynecomastia with or without breast tenderness is a potential side effect of TRT secondary to aromatization of androgens to estradiol which stimulate breast tissue development. Gynecomastia is more commonly seen in elderly men on TRT, probably related to elevated SHBG levels. Though usually transient and may resolves despite continuation of treatment, gynecomastia with breast tenderness can be managed with the addition of antiestrogen such as tamoxifen [114].

4.4.4 Sleep Apnea

Development or worsening of sleep apnea, particularly in obese men or men with chronic obstructive pulmonary disease or smoking history, has been associated with TRT, though most data were from studies using TRT at supraphysiologic doses [115]. Central blunting of CO_2 or increased collapsibility of the upper airway during sleep are some of the suggested mechanisms of sleep apnea exacerbation with TRT [116]. Dose adjustment or discontinuation of TRT or treatment with CPAP for sleep apnea may be considered in managing this complication.

4.4.5 Dermatological Adverse Events

Skin irritation, more commonly with testosterone patch but may rarely occur with other transdermal form, is usually due to skin reaction to the chemicals used for drug delivery. Acne, more common in younger men on TRT, is another dermatological complication secondary to increase secretion of sebum. Management of TRT-induced acne can be managed by good personal hygiene with antiseptic soap. Topical retinoid, benzoyl peroxide, sulfacetamide, or azelaic acid can be

used in more severe cases. Another adverse event of TRT is male pattern baldness that occurs mostly in genetically prone men due to the effects of DHT causing miniaturization on the hair follicles.

4.4.6 Other Adverse Events

Though known breast cancer is an absolute contraindication of TRT, new cases of breast cancer in men treated with TRT remain rare [117]. Flushing of upper body may be due to the action of DHT on the skin and are usually tolerable. Liver toxicity is associated with old testosterone preparation (oral 17-alkylated testosterone derivatives) that is no longer recommended for TRT [19].

4.5 Impact of TRT on Male Reproductive Health

The focus of this section will be on the impact of TRT on male reproductive health through suppression of spermatogenesis. Production of testosterone for clinical use has begun in late 1930s and soon after its effect on male fertility impairment has been recognized [118]. It is thus interesting to see that, in the vast volume of recent publications on hypogonadism management, the negative impact of TRT on male reproductive health is rarely mentioned. Four factors may contribute to this.

First, as mentioned above, testosterone has the effects to enhance sexual function through amelioration of libido or erectile function. Indeed, treatment of sexual dysfunction secondary hypogonadism may lead to increase in frequency of intercourse that is needed for natural fertility. Thus, neither the patients nor treating physicians would intuitively suspect any negative impacts of TRT on male fertility.

Second, many healthcare professionals misunderstood that since testosterone is required for spermatogenesis [119, 120], "extra" testosterone from an exogenous source can only help to further enhance male fertility. Indeed, it is not uncommon to see primary care physicians and gynecologists managing a couple with infertility with low sperm concentration or hypogonadism in the male partners to mistakenly prescribe testosterone hoping to improve their chance of conception. In reality, through negative feedback mechanism, exogenous testosterone will inhibit the release of gonadotropin stimulating hormone and gonadotropins, leading to lack of stimulation of spermatogenesis (and Leydig cells for endogenous androgen production), resulting in impaired fertility and testicular hypo- or atrophy.

Third, there has been a so-called testosterone rebound therapy used since the 1950s for the treatment of idiopathic male infertility [121] in which after testosterone injection therapy resulting in azoospermia, its discontinuation led to increase in semen parameters above baseline with resulting pregnancies. This therapy had misled some clinicians thinking that testosterone is a legitimate treatment option for low sperm concentration. These data, however, did not survive critical assessment and such form of therapy is no longer used since 1980. The observations were likely due to spontaneous fluctuations in semen parameters which, if positive, were wrongly attributed to this "testosterone rebound therapy" [122].

Finally, since the risk of hypogonadism increases with age, most men who are placed on TRT have presumably passed the reproductive age and thus the impact of TRT on spermatogenesis and fertility is considered irrelevant. Indeed, in most interventional studies, even those including subjects below the age of 50 years, semen parameters are generally not part of the outcomes measured. We must, however, keep in mind that in most developed countries, with many couples postponing childbearing until their mid-30s to mid-40s, there is a significant increase in paternal age [123]. Indeed, the birth rates for men aged 20–29 years reached all-time lows in 2009 in the USA while for men aged 40–54 years there has been a steady increase in paternity. Thus, more men who are at risks to develop late-onset hypogonadism and receive TRT will also desire unimpaired fertility, making any potential negative impact of TRT on male reproductive health a timely and relevant health issue.

With the lack of sperm parameters in most interventional studies of TRT on men, our knowledge on the extent of the impact of exogenous testosterone on spermatogenesis is mainly derived from studies on hormonal male contraceptives [124]. The two main functions of testis of testosterone production and spermatogenesis are so closely associated physiologically that it is challenging to interrupt spermatogenesis by hormonal strategies without induction of concomitant hypogonadism with resulting derangement on desirable functions such as libido, potency, and various metabolic processes as described earlier. Exogenous testosterone is, thus, an attractive prototype of hormonal male contraceptive as it can simultaneously suppress gonadotropins to arrest spermatogenesis while maintaining androgenicity.

When extrapolating the results of the various contraceptive studies with native testosterone or other testosterone derivatives to understand their spermatogenic suppression effects, four important points should be kept in mind. First, since native testosterone is rapidly degraded by first-pass metabolism, most of the contraceptive trials relevant to our discussion (i.e., the use of a single testosterone-based drug without combination with other agents such as progestogens) were on chemically modified androgen preparations to achieve a prolonged half-life for a convenient dosing frequency for male contraceptive use. Thus, few studies are done on native testosterone. Second, dosage and/or frequency of the use of these products in these studies may be higher than for general TRT use. Though most contraceptive studies have serum testosterone level monitoring and most subjects had levels within the "normal" range, it is well known that the "normal" serum testosterone range is wide, and most subjects in the trial may be in the higher end of the "normal" range. Third, subjects in these trials tend to be healthy men at younger reproductive ages than typical patients with late-onset hypogonadism requiring TRT. Finally, the availability, contents, and packaging of the various formulations evaluated may vary depending on the legislations of the countries and not all products are necessarily first-line choices of testosterone replacement therapy.

4.5.1 Testosterone Enanthate

Testosterone enanthate was the first testosterone-based product used in large-scale hormonal male contraceptive efficacy study, sponsored by the World Health Organization [125]. An important aspect of many potential male contraceptive methods is that, from the start time of intervention, there is a time lag before a decrease in semen parameters is seen. This lag time occurs for two reasons. First, sperm that have been produced must exit from the testes to the excurrent ductal system and passed by ejaculation. Mechanical contraceptive method like vasectomy is subjected to this lag time. Second, human spermatogenesis cycle of stages may take 2–3 months and therefore, following gonadotropin suppression, a comparable post-intervention lag time is necessary to reach complete spermatogenic suppression. When healthy fertile men were given intramuscularly 200 mg of testosterone enanthate weekly, 70 % reached azoospermia after 6 months. In a subsequent study using 250 mg of testosterone enanthate weekly [126], 98 % of the participants achieved sperm concentration below 3×10^6/mL (taking up to 1 year). For these patients, the contraceptive effect was better than that offered by barrier contraceptive with condoms, with less than two pregnancies per 100 person-year.

4.5.2 Testosterone Buciclate

The World Health Organization's Special Program of Research, Development, and Research Training in Human Reproduction has initiated a testosterone ester synthesis program and identified testosterone buciclate (TB) as the most promising approach to suppression of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Though rarely used for the treatment of hypogonadism at the present time, TB, a long-acting testosterone ester (with a half-life of 29.5 days compared to 4.5 days of testosterone enanthate), can suppress spermatogenesis, reaching azoospermia in three out of eight subjects 10 weeks after a single 1,200 mg injection. Azoospermia has been shown to persist up to 22 weeks [127].

4.5.3 Testosterone Undecanoate

A popular choice of oral formulation for TRT in many countries, testosterone undecanoate was found to suppress spermatogenesis to azoospermia in one out of eight Caucasian subjects at a daily dose of 240 mg over a period of 12 weeks [128]. Due to its short half-life, testosterone undecanoate generally is used orally at multiple daily doses. In a small study with five subjects at 80 mg three times a day for 10–12 weeks, one man became azoospermic, two became oligospermic with sperm concentration below 10×10^6/mL, one had milder degree of sperm concentration decrease, and one showed no change.

Testosterone undecanoate can also be given as 1,000 mg as a depot injection as a TRT. The frequency of dosing is 10–14 week injection intramuscularly. This preparation has been tested as a male hormonal contraceptive at a higher frequency of dosing at 4–8 weeks. In a study on Chinese men with monthly injection of testosterone undeconoate, 11 of the 12 subjects received 500 mg and all 12 subjects of 1,000 mg became azoospermic after 4–6 months of treatment [129]. In a subsequent multicenter efficacy study with over 300 healthy men, 97 % of men achieved azoospermia or severe oligospermia ($<3 \times 10^6$/mL) with an initial loading dose of 1,000 mg followed by monthly 500 mg of testosterone undecanoate injection for 6 months. During another 6 months of efficacy study with continuing monthly 500 mg of testosterone undecanoate injection, only 2 % (6 out of 296) of these subjects had sperm reappear in semen and no pregnancy was achieved. A subsequent 12-month recovery study demonstrated that all subjects had semen parameters returned within the reference range [130]. Though the strong effect of testosterone undecanoate depot injection on suppressing spermatogenesis was further demonstrated in a subsequent phase III clinical trial among Chinese men [131], among Caucasian subject, there appears to be a higher rate of "escape" of complete spermatogenic arrest. Indeed, in an integrated analysis, Liu et al. [132] showed that up to 80 % of Caucasian men vs. up to 90 % of East Asian men suppress sperm output to $<10^6$/mL with androgens, though Caucasian ethnicity predicted faster rates of suppression.

The reasons for the ethnic differences in spermatogenic suppression by testosterone remain speculative [133] and may include: (1) ethnic differences in testicular histomorphometry [134, 135] affecting the intrinsic efficiency of spermatogensis and the response to agents that interfere with the physiological process; (2) differences in hormone concentrations and metabolism of androgen, as demonstrated in various studies [136–146]; (3) differences in CAG- and GGC-polymorphism of the androgen receptor, affecting its activity upon androgen binding [147–150]; and (4) differences in gonadotropin suppressibility [151].

Using 1,000 mg of testosterone undecanoate injection at 6-week interval, 8 of 14 Caucasian subjects achieved azoospermia and an additional 4 of 14 subjects severe oligospermia ($<3 \times 10^6$/mL) at 24 weeks [152]. The authors noted that the extent and kinetics of spermatogenic suppression with injection of 1,000 mg testosterone undecanoate at 6-week intervals is comparable to weekly injection of 200 mg testosterone enanthate. A later pharmacokinetic study concluded that 8-week intervals of 1,000 mg injection would be sufficient for contraceptive purposes [153].

4.5.4 Native Testosterone Pellet

Beside its ester from such as enanthate and undecanoate, native testosterone can also be used as implants inserted surgically under the abdominal skin as a form of TRT to achieve physiological serum testosterone profile with low side effects. McLachlan et al. [154] demonstrated that testosterone implants (800–1,200 mg inserted every 3 months) resulted in suppression of sperm concentration below 1×10^6/mL in 70 % of subjects with no pregnancies ensued over 214 months.

4.5.5 19-Nortestosterone

19-Nortestosterone-hexoxyphenylpropionate represents yet another example of testosterone derivative with longer half-life than testosterone

enanthate as a potential hormonal male contraceptive. Used as anabolic steroid since the 1960s, this 19-nortestosterone ester injected every 3 weeks enabled 10 out of 12 healthy young men to reach azoospermia or severe oligospermia (total sperm count less than 5×10^6) [155], comparable to the effects by testosterone enanthate.

4.5.6 7α-Methyl-19-Nortestosterone

7α-Methyl-19-nortestosterone (MENT) was once considered an ideal option for TRT [156] as it does not undergo 5α-reduction, hence with much lower effect on prostate than on other target organs such as muscle and the pituitary. MENT has tenfold higher potency than testosterone to suppress gonadotropins. In a clinical trial conducted by The Population Council [157] with MENT implant inserted subdermally (each releasing 200–400 μg/day), it was found at 6 months that with two implants inserted, 2 out of 11 subjects became azoospermic and another 2 out of 11 became oligospermic ($<3 \times 10^6$/mL) (none of 12 men with one implant exhibited sperm concentration below 3×10^6/mL). With four implants, 8 of 11 subjects reached azoospermia with one additional subject becoming oligospermic. Upon discontinuation of the drug, subjects with one implant had sperm concentration at or above 20×10^6/mL at 30 days. Recovery time increased at higher doses with a median time to recovery (sperm concentration $>20 \times 10^6$/mL) about 3 months in the four-implant group.

Evidently, there is a considerable risk of spermatogenic suppression with TRT leading to azoospermia, oligospermia, and testicular atrophy, a picture similar to hypogonadotropic hypogonadism. Even for spermatozoa that remain, anomalies in sperm morphology in head and center pieces have been reported in studies on anabolic steroid abuse [158–162]. According to studies on male hormonal contraceptives and anabolic steroid abuse, recovery of spermatogenic function is possible, taking 4–6 months after cessation of TRT but may take up to 3 years or longer [132, 160, 163]. The overall proportion of men recovering spermatogenic function is estimated to be 90 % by 12 months, 96 % by 16 months, and 100 % by 24 months, with East Asian ethnicity predicting a more rapid rate of recovery [132]. Longer treatment studies with more ethnically diverse population (e.g., inclusion of African and Hispanic subjects) are required to fully evaluate the impact of TRT on spermatogenic suppression. For men who clearly desire fertility, treatment of symptomatic hypogonadism with testosterone products should be delayed or avoided. If assisted reproduction is needed, fertility preservation with cryopreservation may be considered before using TRT. Otherwise, various alternative management strategies for late-onset hypogonadism, including lifestyle modification, correction of clinical varicoceles [164], elimination of exposure to drugs and other gonadotoxins, use of antiestrogen or selective estrogen receptor modulator [165, 166], aromatase inhibitors [167], gonadotropin injection [168], or other medical empirical therapies, should be considered when counseling these patients.

4.6 Conclusions

Late-onset hypogonadism is an important men's health issue that has significant negative impact on various aspects of the general health and the quality of life. As the volume of the literature on the various aspects of late-onset hypogonadism and TRT grows, more and more healthcare professionals will adopt an evidence-based approach to diagnose and manage men with the condition. Evidently, questions and controversies do remain on many important aspects of TRT for late-onset hypogonadism, particularly with regard to the various efficacy and safety issues such as the long-term impact of TRT on strength and motor function, prostate cancer risks, improvement in cardiac function, reduction in cardiovascular mortality, and bone fracture rate. Though not frequently included as a point of discussion in most recently published studies on TRT, impairment of male reproductive health through spermatogenic suppression is a timely and relevant issue as men continuing to delay having children until

such age when they are at risk to develop late-onset hypogonadism. Healthcare professionals should, thus, be fully aware of the potential negative health impacts of TRT, in addition to its efficacy, when counseling men presenting with late-onset hypogonadism for the various management options.

References

1. Gooren LJ, Behre HM. Diagnosing and treating testosterone deficiency in different parts of the world: changes between 2006 and 2010. Aging Male. 2012;15(1):22–7.
2. Ramasamy R, Fisher ES, Schlegel PN. Testosterone replacement and prostate cancer. Indian J Urol. 2012;28(2):123–8.
3. Seftel A. Male hypogonadism. Part II: etiology, pathophysiology, and diagnosis. Int J Impot Res. 2006;18(3):223–8. Review.
4. Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. Nat Rev Endocrinol. 2009;5(10):569–76.
5. Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, et al. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. J Clin Endocrinol Metab. 2002;87(2):589–98.
6. Rohrmann S, Platz EA, Selvin E, Shiels MS, Joshu CE, Menke A, et al. The prevalence of low sex steroid hormone concentrations in men in the Third National Health and Nutrition Examination Survey (NHANES III). Clin Endocrinol (Oxf). 2011;75(2):232–9.
7. Araujo AB, O'Donnell AB, Brambilla DJ, Simpson WB, Longcope C, Matsumoto AM, et al. Prevalence and incidence of androgen deficiency in middle-aged and older men: estimates from the Massachusetts Male Aging Study. J Clin Endocrinol Metab. 2004;89(12):5920–6.
8. Mulligan T, Frick MF, Zuraw QC, Stemhagen A, McWhirter C. Prevalence of hypogonadism in males aged at least 45 years: the HIM study. Int J Clin Pract. 2006;60(7):762–9.
9. Kaufman JM, Vermeulen A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev. 2005;26(6):833–76.
10. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2006;295(11):1288–99.
11. Selvin E, Feinleib M, Zhang L, Rohrmann S, Rifai N, Nelson WG, et al. Androgens and diabetes in men: results from the Third National Health and Nutrition Examination Survey (NHANES III). Diabetes Care. 2007;30(2):234–8.
12. Stellato RK, Feldman HA, Hamdy O, Horton ES, McKinlay JB. Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts male aging study. Diabetes Care. 2000;23(4):490–4.
13. Laaksonen DE, Niskanen L, Punnonen K, Nyyssönen K, Tuomainen TP, Valkonen VP, et al. Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. Diabetes Care. 2004;27(5):1036–41.
14. Shores MM, Matsumoto AM, Sloan KL, Kivlahan DR. Low serum testosterone and mortality in male veterans. Arch Intern Med. 2006;166(15):1660–5.
15. Khaw KT, Dowsett M, Folkerd E, Bingham S, Wareham N, Luben R, et al. Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men: European prospective investigation into cancer in Norfolk (EPIC-Norfolk) Prospective Population Study. Circulation. 2007;116(23):2694–701.
16. Moskovic DJ, Araujo AB, Lipshultz LI, Khera M. The 20-year public health impact and direct cost of testosterone deficiency in U.S. men. J Sex Med. 2013;10:562–9.
17. Brown-Sequard CE. The effects produced on man by subcutaneous injection of a liquid obtained from the testicles of animals. Lancet. 1889;137:105–7.
18. Cunningham GR, Toma SM. Clinical review: why is androgen replacement in males controversial? J Clin Endocrinol Metab. 2011;96(1):38–52.
19. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, et al. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2010;95(6):2536–59.
20. Wang C, Nieschlag E, Swerdloff R, Behre HM, Hellstrom WJ, Gooren LJ, et al. ISA, ISSAM, EAU, EAA and ASA recommendations: investigation, treatment and monitoring of late-onset hypogonadism in males. Int J Impot Res. 2009;21(1):1–8.
21. Practice Committee of American Society for Reproductive Medicine in collaboration with Society for Male Reproduction and Urology. Androgen deficiency in the aging male. Fertil Steril. 2008;90(5 Suppl):S83–7.
22. Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Lenrow DA, et al. Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab. 1999;84(8):2647–53.
23. Srinivas-Shankar U, Roberts SA, Connolly MJ, O'Connell MD, Adams JE, Oldham JA, et al. Effects of testosterone on muscle strength, physical function, body composition, and quality of life in intermediate-frail and frail elderly men: a randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab. 2010;95(2):639–50.
24. Kenny AM, Kleppinger A, Annis K, Rathier M, Browner B, Judge JO, et al. Effects of transdermal testosterone on bone and muscle in older men with

low bioavailable testosterone levels, low bone mass, and physical frailty. J Am Geriatr Soc. 2010;58(6): 1134–43.
25. Casaburi R, Bhasin S, Cosentino L, Porszasz J, Somfay A, Lewis MI, et al. Effects of testosterone and resistance training in men with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2004;170(8):870–8.
26. Crawford BA, Liu PY, Kean MT, Bleasel JF, Handelsman DJ. Randomized placebo-controlled trial of androgen effects on muscle and bone in men requiring long-term systemic glucocorticoid treatment. J Clin Endocrinol Metab. 2003;88(7): 3167–76.
27. Basaria S, Coviello AD, Travison TG, Storer TW, Farwell WR, Jette AM, et al. Adverse events associated with testosterone administration. N Engl J Med. 2010;363(2):109–22.
28. Bakhshi V, Elliott M, Gentili A, Godschalk M, Mulligan T. Testosterone improves rehabilitation outcomes in ill older men. J Am Geriatr Soc. 2000;48(5):550–3.
29. Travison TG, Basaria S, Storer TW, Jette AM, Miciek R, Farwell WR, et al. Clinical meaningfulness of the changes in muscle performance and physical function associated with testosterone administration in older men with mobility limitation. J Gerontol A Biol Sci Med Sci. 2011;66(10):1090–9.
30. Emmelot-Vonk MH, Verhaar HJ, Nakhai Pour HR, Aleman A, Lock TM, Bosch JL, et al. Effect of testosterone supplementation on functional mobility, cognition, and other parameters in older men: a randomized controlled trial. JAMA. 2008;299(1): 39–52.
31. Stěpán JJ, Lachman M, Zvěrina J, Pacovský V, Baylink DJ. Castrated men exhibit bone loss: effect of calcitonin treatment on biochemical indices of bone remodeling. J Clin Endocrinol Metab. 1989;69(3):523–7.
32. Benito M, Gomberg B, Wehrli FW, Weening RH, Zemel B, Wright AC, et al. Deterioration of trabecular architecture in hypogonadal men. J Clin Endocrinol Metab. 2003;88(4):1497–502.
33. Meier C, Nguyen TV, Handelsman DJ, Schindler C, Kushnir MM, Rockwood AL, et al. Endogenous sex hormones and incident fracture risk in older men: the Dubbo Osteoporosis Epidemiology Study. Arch Intern Med. 2008;168(1):47–54.
34. LeBlanc ES, Nielson CM, Marshall LM, Lapidus JA, Barrett-Connor E, Ensrud KE, et al. The effects of serum testosterone, estradiol, and sex hormone binding globulin levels on fracture risk in older men. J Clin Endocrinol Metab. 2009;94(9):3337–46.
35. Abbasi AA, Rudman D, Wilson CR, Drinka PJ, Basu SN, Mattson DE, et al. Observations on nursing home residents with a history of hip fracture. Am J Med Sci. 1995;310(6):229–34.
36. Jackson JA, Riggs MW, Spiekerman AM. Testosterone deficiency as a risk factor for hip fractures in men: a case-control study. Am J Med Sci. 1992;304(1):4–8.
37. Stanley HL, Schmitt BP, Poses RM, Deiss WP. Does hypogonadism contribute to the occurrence of a minimal trauma hip fracture in elderly men? J Am Geriatr Soc. 1991;39(8):766–71.
38. Francis RM. Androgen replacement in aging men. Calcif Tissue Int. 2001;69(4):235–8.
39. Aversa A, Bruzziches R, Francomano D, Greco EA, Fornari R, Di Luigi L, et al. Effects of long-acting testosterone undecanoate on bone mineral density in middle-aged men with late-onset hypogonadism and metabolic syndrome: results from a 36 months controlled study. Aging Male. 2012;15(2):96–102.
40. Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto AM, Snyder PJ, et al. Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men. J Clin Endocrinol Metab. 2004;89(5):2085–98.
41. Benito M, Vasilic B, Wehrli FW, Bunker B, Wald M, Gomberg B, et al. Effect of testosterone replacement on trabecular architecture in hypogonadal men. J Bone Miner Res. 2005;20(10):1785–91.
42. Isidori AM, Giannetta E, Greco EA, Gianfrilli D, Bonifacio V, Isidori A, et al. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. Clin Endocrinol (Oxf). 2005;63(3):280–93.
43. Tracz MJ, Sideras K, Boloña ER, Haddad RM, Kennedy CC, Uraga MV, et al. Testosterone use in men and its effects on bone health. A systematic review and meta-analysis of randomized placebo-controlled trials. J Clin Endocrinol Metab. 2006; 91(6):2011–6.
44. Travison TG, Araujo AB, Kupelian V, O'Donnell AB, McKinlay JB. The relative contributions of aging, health, and lifestyle factors to serum testosterone decline in men. J Clin Endocrinol Metab. 2007;92(2):549–55.
45. Rhoden EL, Telöken C, Sogari PR, Souto CA. The relationship of serum testosterone to erectile function in normal aging men. J Urol. 2002;167(4): 1745–8.
46. Marberger M, Wilson TH, Rittmaster RS. Low serum testosterone levels are poor predictors of sexual dysfunction. BJU Int. 2011;108(2):256–62.
47. Kupelian V, Shabsigh R, Travison TG, Page ST, Araujo AB, McKinlay JB. Is there a relationship between sex hormones and erectile dysfunction? Results from the Massachusetts Male Aging Study. J Urol. 2006;176(6 Pt 1):2584–8.
48. Wu FC, Tajar A, Beynon JM, Pye SR, Silman AJ, Finn JD, et al. Identification of late-onset hypogonadism in middle-aged and elderly men. N Engl J Med. 2010;363(2):123–35.
49. Jain P, Rademaker AW, McVary KT. Testosterone supplementation for erectile dysfunction: results of a meta-analysis. J Urol. 2000;164(2):371–5.
50. Boloña ER, Uraga MV, Haddad RM, Tracz MJ, Sideras K, Kennedy CC, et al. Testosterone use in men with sexual dysfunction: a systematic review

and meta-analysis of randomized placebo-controlled trials. Mayo Clin Proc. 2007;82(1):20–8.
51. Isidori AM, Giannetta E, Gianfrilli D, Greco EA, Bonifacio V, Aversa A, et al. Effects of testosterone on sexual function in men: results of a meta-analysis. Clin Endocrinol (Oxf). 2005;63(4):381–94.
52. Rosenthal BD, May NR, Metro MJ, et al. Adjunctive use of AndroGel (testosterone gel) with sildenafil to treat erectile dysfunction in men with acquired androgen deficiency syndrome after failure using sildenafil alone. Urology. 2006;67:571–4.
53. Buvat J, Montorsi F, Maggi M, et al. Hypogonadal men non-responders to the PDE5 inhibitor tadalafil benefit from normalization of testosterone levels with a 1% hydroalcoholic testosterone gel in the treatment of erectile dysfunction (TADTEST study). J Sex Med. 2011;8:284–93.
54. Shamloul R, Ghanem H, Fahmy I, et al. Testosterone therapy can enhance erectile function response to sildenafil in patients with PADAM: a pilot study. J Sex Med. 2005;2:559–64.
55. Hwang TI, Chen HE, Tsai TF, et al. Combined use of androgen and sildenafil for hypogonadal patients non responsive to sildenafil alone. Int J Impot Res. 2006;18:400–4.
56. Shabsigh R, Kaufman JM, Steidle C, et al. Randomized study of testosterone gel as adjunctive therapy to sildenafil in hypogonadal men with erectile dysfunction who do not respond to sildenafil alone. J Urol. 2004;172:658–63.
57. Barrett-Connor E, Von Mühlen DG, Kritz-Silverstein D. Bioavailable testosterone and depressed mood in older men: the Rancho Bernardo Study. J Clin Endocrinol Metab. 1999;84(2):573–7.
58. Moffat SD, Zonderman AB, Metter EJ, Kawas C, Blackman MR, Harman SM, et al. Free testosterone and risk for Alzheimer disease in older men. Neurology. 2004;62(2):188–93.
59. Gouras GK, Xu H, Gross RS, Greenfield JP, Hai B, Wang R, et al. Testosterone reduces neuronal secretion of Alzheimer's beta-amyloid peptides. Proc Natl Acad Sci USA. 2000;97(3):1202–5.
60. Zarrouf FA, Artz S, Griffith J, Sirbu C, Kommor M. Testosterone and depression: systematic review and meta-analysis. J Psychiatr Pract. 2009;15(4): 289–305.
61. Wang C, Swerdloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, et al. Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. J Clin Endocrinol Metab. 2000;85(8):2839–53.
62. Pope Jr HG, Amiaz R, Brennan BP, Orr G, Weiser M, Kelly JF, et al. Parallel-group placebo-controlled trial of testosterone gel in men with major depressive disorder displaying an incomplete response to standard antidepressant treatment. J Clin Psychopharmacol. 2010;30(2):126–34.
63. Behre HM, Tammela TL, Arver S, Tolrá JR, Bonifacio V, Lamche M, et al. A randomized, double-blind, placebo-controlled trial of testosterone gel on body composition and health-related quality-of-life in men with hypogonadal to low-normal levels of serum testosterone and symptoms of androgen deficiency over 6 months with 12 months open-label follow-up. Aging Male. 2012;15(4):198–207.
64. Zhang XW, Liu ZH, Hu XW, Yuan YQ, Bai WJ, Wang XF, et al. Androgen replacement therapy improves psychological distress and health-related quality of life in late onset hypogonadism patients in Chinese population. Chin Med J (Engl). 2012; 125(21):3806–10.
65. Ho CC, Tong SF, Low WY, Ng CJ, Khoo EM, Lee VK, et al. A randomized, double-blind, placebo-controlled trial on the effect of long-acting testosterone treatment as assessed by the Aging Male Symptoms scale. BJU Int. 2012;110(2):260–5.
66. Tong SF, Ng CJ, Lee BC, Lee VK, Khoo EM, Lee EG, et al. Effect of long-acting testosterone undecanoate treatment on quality of life in men with testosterone deficiency syndrome: a double blind randomized controlled trial. Asian J Androl. 2012; 14(4):604–11.
67. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome–a new world-wide definition. A consensus statement from the International Diabetes Federation. Diabet Med. 2006;23(5):469–80.
68. Corona G, Rastrelli G, Morelli A, Vignozzi L, Mannucci E, Maggi M. Hypogonadism and metabolic syndrome. J Endocrinol Invest. 2011;34(7): 557–67.
69. Corona G, Monami M, Rastrelli G, Aversa A, Tishova Y, Saad F, et al. Testosterone and metabolic syndrome: a meta-analysis study. J Sex Med. 2011;8(1):272–83.
70. Whitsel EA, Boyko EJ, Matsumoto AM, Anawalt BD, Siscovick DS. Intramuscular testosterone esters and plasma lipids in hypogonadal men: a meta-analysis. Am J Med. 2001;111(4):261–9.
71. Corona G, Monami M, Rastrelli G, Aversa A, Sforza A, Lenzi A, et al. Type 2 diabetes mellitus and testosterone: a meta-analysis study. Int J Androl. 2011;34 (6 Pt 1):528–40.
72. Cherrier MM, Asthana S, Plymate S, Baker L, Matsumoto AM, Peskind E, et al. Testosterone supplementation improves spatial and verbal memory in healthy older men. Neurology. 2001;57(1):80–8.
73. Cherrier MM, Plymate S, Mohan S, Asthana S, Matsumoto AM, Bremner W, et al. Relationship between testosterone supplementation and insulin-like growth factor-I levels and cognition in healthy older men. Psychoneuroendocrinology. 2004;29(1):65–82.
74. Cherrier MM, Matsumoto AM, Amory JK, Asthana S, Bremner W, Peskind ER, et al. Testosterone improves spatial memory in men with Alzheimer disease and mild cognitive impairment. Neurology. 2005;64(12):2063–8.
75. Haren MT, Wittert GA, Chapman IM, Coates P, Morley JE. Effect of oral testosterone undecanoate on visuospatial cognition, mood and quality of life in elderly men with low-normal gonadal status. Maturitas. 2005;50(2):124–33.

76. Corona G, Rastrelli G, Vignozzi L, Mannucci E, Maggi M. Testosterone, cardiovascular disease and the metabolic syndrome. Best Pract Res Clin Endocrinol Metab. 2011;25(2):337–53.
77. Corona G, Monami M, Boddi V, Cameron-Smith M, Fisher AD, de Vita G, et al. Low testosterone is associated with an increased risk of MACE lethality in subjects with erectile dysfunction. J Sex Med. 2010;7(4 Pt 1):1557–64.
78. Corona G, Rastrelli G, Monami M, Guay A, Buvat J, Sforza A, et al. Hypogonadism as a risk factor for cardiovascular mortality in men: a meta-analytic study. Eur J Endocrinol. 2011;165(5):687–701. doi:10.1530/EJE-11-0447.
79. Malkin CJ, Pugh PJ, Morris PD, Asif S, Jones TH, Channer KS. Low serum testosterone and increased mortality in men with coronary heart disease. Heart. 2010;96(22):1821–5.
80. Jones TH. Testosterone deficiency: a risk factor for cardiovascular disease? Trends Endocrinol Metab. 2010;21(8):496–503.
81. Corona G, Rastrelli G, Forti G, Maggi M. Update in testosterone therapy for men. J Sex Med. 2011; 8(3):639–54.
82. Saad F. Androgen therapy in men with testosterone deficiency: can testosterone reduce the risk of cardiovascular disease? Diabetes Metab Res Rev. 2012;28 Suppl 2:52–9.
83. Fernández-Balsells MM, Murad MH, Lane M, Lampropulos JF, Albuquerque F, Mullan RJ, et al. Clinical review 1: adverse effects of testosterone therapy in adult men: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2010;95(6): 2560–75.
84. Haddad RM, Kennedy CC, Caples SM, Tracz MJ, Boloña ER, Sideras K, et al. Testosterone and cardiovascular risk in men: a systematic review and meta-analysis of randomized placebo-controlled trials. Mayo Clin Proc. 2007;82(1):29–39.
85. Calof OM, Singh AB, Lee ML, Kenny AM, Urban RJ, Tenover JL, et al. Adverse events associated with testosterone replacement in middle-aged and older men: a meta-analysis of randomized, placebo-controlled trials. J Gerontol A Biol Sci Med Sci. 2005;60(11):1451–7.
86. Morris PD, Channer KS. Testosterone and cardiovascular disease in men. Asian J Androl. 2012; 14(3):428–35.
87. Saad F, Aversa A, Isidori AM, Zafalon L, Zitzmann M, Gooren L. Onset of effects of testosterone treatment and time span until maximum effects are achieved. Eur J Endocrinol. 2011;165(5):675–85.
88. Morgentaler A. Words of wisdom. Re: adverse events associated with testosterone administration. Eur Urol. 2011;59(3):465.
89. Favilla V, Cimino S, Castelli T, Madonia M, Barbagallo I, Morgia G. Relationship between lower urinary tract symptoms and serum levels of sex hormones in men with symptomatic benign prostatic hyperplasia. BJU Int. 2010;106(11):1700–3.
90. Litman HJ, Bhasin S, O'Leary MP, Link CL, McKinlay JB, BACH Survey Investigators. An investigation of the relationship between sex-steroid levels and urological symptoms: results from the Boston Area Community Health survey. BJU Int. 2007;100(2):321–6.
91. Rohrmann S, Nelson WG, Rifai N, Kanarek N, Basaria S, Tsilidis KK, et al. Serum sex steroid hormones and lower urinary tract symptoms in Third National Health and Nutrition Examination Survey (NHANES III). Urology. 2007;69(4):708–13.
92. Liao CH, Chiang HS, Yu HJ. Serum testosterone levels significantly correlate with nocturia in men aged 40-79 years. Urology. 2011;78(3):631–5.
93. Polackwich AS, Ostrowski KA, Hedges JC. Testosterone replacement therapy and prostate health. Curr Urol Rep. 2012;13(6):441–6.
94. Trifiro MD, Parsons JK, Palazzi-Churas K, Bergstrom J, Lakin C, Barrett-Connor E. Serum sex hormones and the 20-year risk of lower urinary tract symptoms in community-dwelling older men. BJU Int. 2010;105(11):1554–9.
95. Shigehara K, Namiki M. Late-onset hypogonadism syndrome and lower urinary tract symptoms. Korean J Urol. 2011;52(10):657–63.
96. Takao T, Tsujimura A, Okuda H, Yamamoto K, Fukuhara S, Matsuoka Y, et al. Lower urinary tract symptoms and erectile dysfunction associated with depression among Japanese patients with late-onset hypogonadism symptoms. Aging Male. 2011;14(2):110–4.
97. Haider A, Gooren LJ, Padungtod P, Saad F. Concurrent improvement of the metabolic syndrome and lower urinary tract symptoms upon normalisation of plasma testosterone levels in hypogonadal elderly men. Andrologia. 2009;41(1):7–13.
98. Pechersky AV, Mazurov VI, Semiglazov VF, Karpischenko AI, Mikhailichenko VV, Udintsev AV. Androgen administration in middle-aged and ageing men: effects of oral testosterone undecanoate on dihydrotestosterone, oestradiol and prostate volume. Int J Androl. 2002;25(2):119–25.
99. Karazindiyano lu S, Cayan S. The effect of testosterone therapy on lower urinary tract symptoms/bladder and sexual functions in men with symptomatic late-onset hypogonadism. Aging Male. 2008;11(3):146–9.
100. Kalinchenko S, Vishnevskiy EL, Koval AN, Mskhalaya GJ, Saad F. Beneficial effects of testosterone administration on symptoms of the lower urinary tract in men with late-onset hypogonadism: a pilot study. Aging Male. 2008;11(2):57–61.
101. Amano T, Imao T, Takemae K, Iwamoto T, Nakanome M. Testosterone replacement therapy by testosterone ointment relieves lower urinary tract symptoms in late onset hypogonadism patients. Aging Male. 2010;13(4):242–6.
102. Shigehara K, Sugimoto K, Konaka H, Iijima M, Fukushima M, Maeda Y, et al. Androgen replacement therapy contributes to improving lower urinary tract symptoms in patients with hypogonadism and

benign prostate hypertrophy: a randomised controlled study. Aging Male. 2011;14(1):53–8.
103. Huggins C, Steven RE, Hodges CV. Studies on prostatic cancer. Arch Surg. 1941;43:209–23.
104. Morgentaler A. Testosterone replacement therapy and prostate cancer. Urol Clin North Am. 2007;34(4):555–63.
105. Marks LS, Mazer NA, Mostaghel E, Hess DL, Dorey FJ, Epstein JI, et al. Effect of testosterone replacement therapy on prostate tissue in men with late-onset hypogonadism: a randomized controlled trial. JAMA. 2006;296(19):2351–61.
106. Raynaud JP, Gardette J, Rollet J, Legros JJ. Prostate-specific antigen (PSA) concentrations in hypogonadal men during 6 years of transdermal testosterone treatment. BJU Int. 2013 Jan 7. doi: 10.1111/j.1464-410X.2012.11514.x. [Epub ahead of print]. PMID: 23294726.
107. Kaufman JM, Graydon RJ. Androgen replacement after curative radical prostatectomy for prostate cancer in hypogonadal men. J Urol. 2004;172(3):920–2.
108. Agarwal PK, Oefelein MG. Testosterone replacement therapy after primary treatment for prostate cancer. J Urol. 2005;173(2):533–6.
109. Khera M, Grober ED, Najari B, Colen JS, Mohamed O, Lamb DJ, et al. Testosterone replacement therapy following radical prostatectomy. J Sex Med. 2009;6(4):1165–70.
110. Morales A, Black AM, Emerson LE. Testosterone administration to men with testosterone deficiency syndrome after external beam radiotherapy for localized prostate cancer: preliminary observations. BJU Int. 2009;103(1):62–4.
111. Pastuszak AW, Pearlman AM, Godoy G, Miles BJ, Lipshultz LI, Khera M. Testosterone replacement therapy in the setting of prostate cancer treated with radiation. Int J Impot Res. 2013;25(1):24–8.
112. Sarosdy MF. Testosterone replacement for hypogonadism after treatment of early prostate cancer with brachytherapy. Cancer. 2007;109(3):536–41.
113. Morgentaler A. Testosterone therapy in men with prostate cancer: scientific and ethical considerations. J Urol. 2013;189(1 Suppl):S26–33.
114. Braunstein GD. Clinical practice. Gynecomastia. N Engl J Med. 2007;357(12):1229–37.
115. Hanafy HM. Testosterone therapy and obstructive sleep apnea: is there a real connection? J Sex Med. 2007;4(5):1241–6.
116. Basaria S, Dobs AS. Hypogonadism and androgen replacement therapy in elderly men. Am J Med. 2001;110(7):563–72.
117. Thomas SR, Evans PJ, Holland PA, Biswas M. Invasive breast cancer after initiation of testosterone replacement therapy in a man–a warning to endocrinologists. Endocr Pract. 2008;14(2):201–3.
118. Heckel M. Production of oligospermia in a man by the use of testosterone propionate. Proc Soc Exp Biol Med. 1939;40:658–9.
119. Ruwanpura SM, McLachlan RI, Meachem SJ. Hormonal regulation of male germ cell development. J Endocrinol. 2010;205(2):117–31.
120. Walker WH. Testosterone signaling and the regulation of spermatogenesis. Spermatogenesis. 2011;1(2):116–20.
121. Heckel NJ, Rosso WA, Kestel L. Spermatogenic rebound phenomenon after administration of testosterone propionate. J Clin Endocrinol Metab. 1951;11(3):235–45.
122. Nieschlag E, Vorona E, Wenk M, Hemker AK, Kamischke A, Zitzmann M. Hormonal male contraception in men with normal and subnormal semen parameters. Int J Androl. 2011;34(6 Pt 1):556–67.
123. Martin JA, Hamilton BE, Ventura SJ, Osterman MJ, Kirmeyer S, Mathews TJ, et al. Births: final data for 2009. Natl Vital Stat Rep. 2011;60(1):1–70.
124. Nieschlag E. Clinical trials in male hormonal contraception. Contraception. 2010;82(5):457–70.
125. World Health Organization. Contraceptive efficacy of testosterone-induced azoospermia in normal men. World Health Organization Task Force on methods for the regulation of male fertility. Lancet. 1990;336(8721):955–9.
126. World Health Organization. Contraceptive efficacy of testosterone-induced azoospermia and oligozoospermia in normal men. Fertil Steril. 1996;65(4):821–9.
127. Behre HM, Baus S, Kliesch S, Keck C, Simoni M, Nieschlag E. Potential of testosterone buciclate for male contraception: endocrine differences between responders and nonresponders. J Clin Endocrinol Metab. 1995;80(8):2394–403.
128. Nieschlag E, Hoogen H, Bölk M, Schuster H, Wickings EJ. Clinical trial with testosterone undecanoate for male fertility control. Contraception. 1978;18(6):607–14.
129. Zhang GY, Gu YQ, Wang XH, Cui YG, Bremner WJ. A clinical trial of injectable testosterone undecanoate as a potential male contraceptive in normal Chinese men. J Clin Endocrinol Metab. 1999;84(10):3642–7.
130. Gu YQ, Wang XH, Xu D, Peng L, Cheng LF, Huang MK, et al. A multicenter contraceptive efficacy study of injectable testosterone undecanoate in healthy Chinese men. J Clin Endocrinol Metab. 2003;88(2):562–8.
131. Gu Y, Liang X, Wu W, Liu M, Song S, Cheng L, et al. Multicenter contraceptive efficacy trial of injectable testosterone undecanoate in Chinese men. J Clin Endocrinol Metab. 2009;94(6):1910–5.
132. Liu PY, Swerdloff RS, Anawalt BD, Anderson RA, Bremner WJ, Elliesen J, et al. Determinants of the rate and extent of spermatogenic suppression during hormonal male contraception: an integrated analysis. J Clin Endocrinol Metab. 2008;93(5):1774–83.
133. Ilani N, Liu PY, Swerdloff RS, Wang C. Does ethnicity matter in male hormonal contraceptive efficacy? Asian J Androl. 2011;13(4):579–84.
134. Johnson L, Barnard JJ, Rodriguez L, Smith EC, Swerdloff RS, Wang XH, et al. Ethnic differences in testicular structure and spermatogenic potential may predispose testes of Asian men to a heightened sensitivity to steroidal contraceptives. J Androl. 1998;19(3):348–57.
135. Hikim AP, Wang C, Lue Y, Johnson L, Wang XH, Swerdloff RS. Spontaneous germ cell apoptosis in

humans: evidence for ethnic differences in the susceptibility of germ cells to programmed cell death. J Clin Endocrinol Metab. 1998;83(1):152–6.
136. Santner SJ, Albertson B, Zhang GY, Zhang GH, Santulli M, et al. Comparative rates of androgen production and metabolism in Caucasian and Chinese subjects. J Clin Endocrinol Metab. 1998;83:2104–9.
137. Wang C, Catlin DH, Starcevic B, Leung A, DiStefano E, et al. Testosterone metabolic clearance and production rates determined by stable isotope dilution/tandem mass spectrometry in normal men: influence of ethnicity and age. J Clin Endocrinol Metab. 2004;89:2936–41.
138. Lookingbill DP, Demers LM, Wang C, Leung A, Rittmaster RS, et al. Clinical and biochemical parameters of androgen action in normal healthy Caucasian versus Chinese subjects. J Clin Endocrinol Metab. 1991;72:1242–8.
139. Wu AH, Whittemore AS, Kolonel LN, John EM, Gallagher RP, et al. Serum androgens and sex hormone-binding globulins in relation to lifestyle factors in older African-American, white, and Asian men in the United States and Canada. Cancer Epidemiol Biomarkers Prev. 1995;4:735–41.
140. Ross RK, Bernstein L, Lobo RA, Shimizu H, Stanczyk FZ, et al. 5-alpha-reductase activity and risk of prostate cancer among Japanese and US white and black males. Lancet. 1992;339:887–9.
141. Orwoll ES, Nielson CM, Labrie F, Barrett-Connor E, Cauley JA, et al. Evidence for geographical and racial variation in serum sex steroid levels in older men. J Clin Endocrinol Metab. 2010;95:E151–60.
142. Schulze JJ, Lundmark J, Garle M, Skilving I, Ekstrom L, et al. Doping test results dependent on genotype of uridine diphospho-glucuronosyl transferase 2B17, the major enzyme for testosterone glucuronidation. J Clin Endocrinol Metab. 2008;93:2500–6.
143. Swanson C, Mellstrom D, Lorentzon M, Vandenput L, Jakobsson J, et al. The uridine diphosphate glucuronosyltransferase 2B15 D85Y and 2B17 deletion polymorphisms predict the glucuronidation pattern of androgens and fat mass in men. J Clin Endocrinol Metab. 2007;92:4878–82.
144. Jakobsson J, Ekstrom L, Inotsume N, Garle M, Lorentzon M, et al. Large differences in testosterone excretion in Korean and Swedish men are strongly associated with a UDP-glucuronosyl transferase 2B17 polymorphism. J Clin Endocrinol Metab. 2006;91:687–93.
145. Vandenput L, Labrie F, Mellstrom D, Swanson C, Knutsson T, et al. Serum levels of specific glucuronidated androgen metabolites predict BMD and prostate volume in elderly men. J Bone Miner Res. 2007;22:220–7.
146. Xue Y, Sun D, Daly A, Yang F, Zhou X, et al. Adaptive evolution of UGT2B17 copy-number variation. Am J Hum Genet. 2008;83:337–46.
147. Giovannucci E, Stampfer MJ, Krithivas K, Brown M, Dahl D, et al. The CAG repeat within the androgen receptor gene and its relationship to prostate cancer [published erratum appears in Proc Natl Acad Sci USA 1997;94(15):8272]. Proc Natl Acad Sci USA. 1997;94:3320–3.
148. Esteban E, Rodon N, Via M, Gonzalez-Perez E, Santamaria J, et al. Androgen receptor CAG and GGC polymorphisms in Mediterraneans: repeat dynamics and population relationships. J Hum Genet. 2006;51:129–36.
149. Eckardstein SV, Schmidt A, Kamischke A, Simoni M, Gromoll J, et al. CAG repeat length in the androgen receptor gene and gonadotrophin suppression influence the effectiveness of hormonal male contraception. Clin Endocrinol (Oxf). 2002;57:647–55.
150. Li JW, Gu YQ. Predictors for partial suppression of spermatogenesis of hormonal male contraception. Asian J Androl. 2008;10:723–30.
151. Wang C, Berman NG, Veldhuis JD, Der T, McDonald V, et al. Graded testosterone infusions distinguish gonadotropin negative-feedback responsiveness in Asian and white men—a Clinical Research Center study. J Clin Endocrinol Metab. 1998;83:870–6.
152. Kamischke A, Plöger D, Venherm S, von Eckardstein S, von Eckardstein A, Nieschlag E. Intramuscular testosterone undecanoate with or without oral levonorgestrel: a randomized placebo-controlled feasibility study for male contraception. Clin Endocrinol (Oxf). 2000;53(1):43–52.
153. Qoubaitary A, Meriggiola C, Ng CM, Lumbreras L, Cerpolini S, Pelusi G, et al. Pharmacokinetics of testosterone undecanoate injected alone or in combination with norethisterone enanthate in healthy men. J Androl. 2006;27(6):853–67.
154. McLachlan RI, McDonald J, Rushford D, Robertson DM, Garrett C, Baker HW. Efficacy and acceptability of testosterone implants, alone or in combination with a 5alpha-reductase inhibitor, for male hormonal contraception. Contraception. 2000;62(2):73–8.
155. Knuth UA, Behre H, Belkien L, Bents H, Nieschlag E. Clinical trial of 19-nortestosterone-hexoxyphenylpropionate (Anadur) for male fertility regulation. Fertil Steril. 1985;44(6):814–21.
156. Sundaram K, Kumar N, Bardin CW. 7 alpha-Methyl-19-nortestosterone: an ideal androgen for replacement therapy. Recent Prog Horm Res. 1994;49:373–6.
157. von Eckardstein S, Noe G, Brache V, Nieschlag E, Croxatto H, Alvarez F, et al. A clinical trial of 7 alpha-methyl-19-nortestosterone implants for possible use as a long-acting contraceptive for men. J Clin Endocrinol Metab. 2003;88(11):5232–9.
158. Bonetti A, Tirelli F, Catapano A, Dazzi D, Dei Cas A, Solito F, et al. Side effects of anabolic androgenic steroids abuse. Int J Sports Med. 1996;17(4):259–62.
159. Knuth UA, Maniera H, Nieschlag E. Anabolic steroids and semen parameters in bodybuilders. Fertil Steril. 1989;52(6):1041–7.
160. Schürmeyer T, Knuth UA, Belkien L, Nieschlag E. Reversible azoospermia induced by the anabolic steroid 19-nortestosterone. Lancet. 1984;1(8374):417–20.
161. Torres-Calleja J, González-Unzaga M, DeCelis-Carrillo R, Calzada-Sánchez L, Pedrón N. Effect of

androgenic anabolic steroids on sperm quality and serum hormone levels in adult male bodybuilders. Life Sci. 2001;68(15):1769–74.
162. Moretti E, Collodel G, La Marca A, Piomboni P, Scapigliati G, Baccetti B. Structural sperm and aneuploidies studies in a case of spermatogenesis recovery after the use of androgenic anabolic steroids. J Assist Reprod Genet. 2007;24:195–8.
163. Turek PJ, Williams RH, Gilbaugh 3rd JH, Lipshultz LI. The reversibility of anabolic steroid-induced azoospermia. J Urol. 1995;153(5):1628–30.
164. Tanrikut C, McQuaid JW, Goldstein M. The impact of varicocele and varicocele repair on serum testosterone. Curr Opin Obstet Gynecol. 2011; 23(4):227–31.
165. Tsourdi E, Kourtis A, Farmakiotis D, Katsikis I, Salmas M, Panidis D. The effect of selective estrogen receptor modulator administration on the hypothalamic-pituitary-testicular axis in men with idiopathic oligozoospermia. Fertil Steril. 2009;91 (4 Suppl):1427–30.
166. Corona G, Rastrelli G, Vignozzi L, Maggi M. Emerging medication for the treatment of male hypogonadism. Expert Opin Emerg Drugs. 2012; 17(2):239–59.
167. Gregoriou O, Bakas P, Grigoriadis C, Creatsa M, Hassiakos D, Creatsas G. Changes in hormonal profile and seminal parameters with use of aromatase inhibitors in management of infertile men with low testosterone to estradiol ratios. Fertil Steril. 2012;98(1):48–51.
168. Hsieh TC, Pastuszak AW, Hwang K, Lipshultz LI. Concomitant intramuscular human chorionic gonadotropin preserves spermatogenesis in men undergoing testosterone replacement therapy. J Urol. 2013;189(2):647–50.

Part II

Female Infertility

A Practical Approach to Recent Advances in Ovarian Reserve Testing

5

Benjamin Leader and Valerie L. Baker

"It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity…"
Charles Dickens, A Tale of Two Cities

5.1 Introduction

5.1.1 Progress

For pre and perimenopausal women, current clinically available ORTs provide important new benefits (Table 5.1) which primarily has been driven for the last decade by advancements in clinical research, much of which incorporates the use of antral follicle count (AFC) and serum biomarkers such as anti-mullerian hormone (AMH). In the field of assisted reproductive technologies (ART), progress with ORT clinical research has led to improved clinical practice with respect to prediction of ovulatory response [1–6] and optimization of oocyte retrieval using ORTs that can help more efficiently dose medications [7] and minimize side effects such as ovarian hyperstimulation syndrome (OHSS) [8, 9]. Although not ready for routine, general use, under appropriate guidance by a fertility specialist, women can now obtain noninvasive, widely accessible ORTs that provide clinically useful general information regarding their egg supply and likelihood of menopause being earlier relative to the population average [10–13]. The recent advances with ORTs have far reaching implications for improvements in medical care by earlier detection of primary ovarian insufficiency (POI) and polycystic ovary syndrome (PCOS), counseling regarding use of fertility preservation, assessment of ovarian injury via surgery or medications such as chemotherapy, and monitoring of ovarian-related cancers [14]. These advances can, therefore, directly improve the quality of life for many women and their partners through better medical management as well as more informed decision making across a wide spectrum of medical topics.

B. Leader, M.D., Ph.D. (✉)
Clinical Research Division, ReproSource Inc., 300 Trade Center, Suite 6540, Woburn, MA 01801, USA
e-mail: leader@reprosource.com

V.L. Baker, M.D.
Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Stanford University School of Medicine, Palo Alto, CA, USA

5.1.2 Challenges

Although correctly interpreted ORT results currently have great potential to benefit patients, there remains a significant risk that the results may be misinterpreted either by the clinician or

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_5,

Table 5.1 Overview of clinical applications of ORTs

ORT clinical application	Clinical outcomes assessed	Current clinical uses	Limitations	Research applications
Predict response to COS	Follicular response, # of oocyte retrieved, cycle cancelation, excessive response/ OHSS	Used to modify COS medication/protocol to reduce incidence of hyper-response, counsel poor responders	No consensus, Site-specific cut points, protocols	Optimize number of oocytes retrieved and medication dosing, improve cost effectiveness, and reduce adverse reactions
Oocyte quality, ART success	Rates of fertilization, blastocyst formation, implantation, live birth	Counsel patients about likelihood of success, which is program specific	Variable approaches, strong disagreement as to clinical value of ORT use	Algorithms to establish individualized probability estimates and improve success
Natural fertility	Live birth after attempts at natural conception	At best, inform women of possible increased risk of infertility	Thresholds not available for routine clinical use	Identifying who is at risk for being infertile, predict current fertility, and fertility window
PCOS risk/ diagnosis	PCOS diagnosis via Rotterdam criteria	Further PCOS eval for high risk, used by some in diagnosis if APC unavailable	No agreed upon thresholds	PCOS-specific treatment protocols
Primary Ovarian insufficiency	Menstrual irregularities or absence, infertility, poor response to COs	If abnormal proactive fertility assessments, planning of future reproductive attempts	Thresholds not available for routine screening	Establish screening protocol with associated medical care algorithms
Menopause Prediction	Last menstrual period, menopausal staging criteria	Qualitative information only regarding possibly increased risk of menopause earlier than average	Specific time estimates not ready for clinical use	Establish accurate and specific predication of time to perimenopause and last menstrual period

the patient. Furthermore, the medical community remains far from reaching consensus regarding the use of ORTs [15]. The causes for this concerning lack of consensus can be grouped into two major areas: (1) lack of standardization and (2) lack of tests that can assess egg quality. In the generation and application of ORT results, major challenges exist in standardizing testing materials and methods and in widespread definitional differences used in research and clinical care with respect to the phenotypes of patients tested, medical indications, clinical outcomes managed, and selected diagnostic cut points. Overcoming these challenges is further hampered by the testing technology itself which currently only demonstrates strong prediction of oocyte quantity not oocyte quality, both of which are needed for full assessment of ovarian reserve and chances of pregnancy. Underlying the difficulty in connecting ORT results to oocyte quality is that oocyte quality ultimately is proven by the success of an oocyte to develop into a healthy baby which is a process that requires many other factors in addition to oocyte quality.

5.2 Definitions

5.2.1 Ovarian Reserve

A woman's reproductive potential, as determined by her oocyte quantity and quality, is often defined as her ovarian reserve. Although multiple factors contribute to a woman's ability to have a baby, an assessment of ovarian reserve allows approximation of a woman's fertility potential as it relates to the contribution from her oocytes. However, currently, no test can definitively determine how many oocytes a woman has and/ or which oocytes are capable of conceiving an embryo that can become a healthy baby. Therefore, ovarian reserve functionally is defined in the literature by those clinical outcomes that can be measured. The advent of the ART field has provided an artificial circumstance that allows measurement of a wide variety of clinical outcome parameters not available for measure in natural reproduction. In fact, until recently [16, 17], available tests of ovarian reserve were

not validated to any natural fertility parameter but were mainly calibrated to surrogates of only ovarian quantity obtained from ART treatment outcomes, such as oocytes retrieved through controlled ovarian stimulation (COS). Although future studies may prove otherwise, when compared to age alone, ORTs have not consistently demonstrated a substantially superior ability to predict chance of spontaneous conception or live birth rate with fertility treatment.

5.2.2 Oocyte Quantity

Although recently a question has been raised as to whether human oocytes may be regenerated later in life [18], most data support the concept that oocyte supply is set at birth and is depleted over time [19]. Ironically, as the number of oocytes is not actually measurable directly without removing and dissecting the ovaries, clinical measurements of oocyte quantity are defined qualitatively. Sonographic assessment of the number of growing follicles appears to correlate with the total number of oocytes as quantified histologically [20]. Another quantifiable, clinically available measure of oocyte quantity is the number of oocytes retrieved through COS. In order for response to COS to provide a reasonable assessment of ovarian reserve, gonadotropins must be administered at doses chosen to achieve an oocyte number that maximizes live birth rate without undue risk of OHSS. The number of oocytes retrieved during an IVF attempt functions as a surrogate to approximate the number of remaining oocytes in woman [4, 6, 21–24].

5.2.3 Oocyte Quality

Oocyte quality generally refers to the ability of an oocyte to perform its primary function: to produce a healthy baby in conjunction with the genetic material supplied by a sperm. However, the creation of a healthy baby involves a multitude of factors such that the oocyte plays the classic scientific "necessary but not sufficient" role. It is currently difficult to independently measure and accurately quantify the non-oocyte contributions that are required to have a healthy baby such as sperm or endometrial quality. Furthermore, when fewer live births occur than embryos transferred to the recipient woman, it has historically not been possible to definitively link an individual oocyte to the individual baby born. Recently, however, the increased use of elective single embryo transfer and "genetic fingerprinting" of each embryo prior to transfer, allows individual assessments of oocytes or embryos to be linked to their outcome [25–27]. Currently, oocyte quality for a woman is not assessed at the individual oocyte level but generally is inferred from calculated rates or averages from clinical endpoints such as fertilization rate, blastocyst formation rate, morphologic assessment of embryo quality, implantation rate, and live birth rate.

5.2.4 Cumulative Live Birth Rate/Total Reproductive Potential

As a concept, the number of oocytes avialable that are capable of producing a healthy baby can be further larger concepts such as cumulative live birth rate or total reproductive potential. There is a growing sentiment that the live birth rate per cycle has perhaps been overemphasized as a measure of fertility treatment success, and instead perhaps more focus should be placed on the cumulative chance of live birth rate over a course of treatment which may include multiple cycles of intrauterine insemination or multiple fresh and frozen cycles of IVF [28]. The term "total reproductive potential" has been introduced and is defined as the chance of live birth from one ovarian stimulation and oocyte retrieval, including the pregnancies from all fresh and all frozen embryo transfers associated with this ovarian stimulation [29]. Nearly all publications to date which have examined the prognostic value of ORTs for IVF cycles have focused on the live birth rate from one fresh transfer, not the cumulative live birth rate over multiple fresh and frozen cycles, nor the total reproductive potential from a single cycle. It would be valuable that future studies examining the prognostic value of ORTs also assess cumulative live birth rate and/or total reproductive potential as outcomes of interest.

Table 5.2 Qualitative overview of ORT correlation strength to various clinical outcomes

ORT	Modality	Response to COS	Live birth rate	Natural fertility	PCOS	POI	Menopause	Comment
AFC	Imaging	++	+	+	++	++	+	Widely available in ART centers. Highly user dependent, although automated systems may reduce user variability. Obesity may limit use
AMH	Biomarker	++	+	+	++	++	++	Measure across menstrual cycle. Can vary significantly within individuals, although less than other ORTs. Multiple charging diagnostic platforms in past and in future with no reference materials
FSH	Biomarker	+	+	+	+	++	++	High cut points identify poor response or success in a small percentage of patients but with poor sensitivity
Inhibin B	Biomarker	+/−	−	−	−	+	+	Confirmatory of other markers. Recent change in available diagnostic platforms associated with poor performance in many studies
CCCT/ EFFORT (rise in FSH/Inh B)	Patient response	++	+	−	−	+	−	Can increase predictive performance of FSH and Inhibin B. Requires two measurements and medication dose with modest additive information to single marker such as AMH or AFC. Infrequently used
Outcome of Prior ART cycle	Patient response	++	++	−	−	++	+	Not possible to use in initial assessment. Stronger data in older patients
Combinations	Multivariate	+	+	−	−	−	++	Heterogeneity of study design and ORTs prevent meaningful comparisons. Published studies show at best modest improvements currently but with continuing improvement

++ multiple published studies from multiple sources supporting strong correlation; + some evidence establishing a correlation with possible contradictory results in other studies; +/− recent studies not supportive of association; − insufficient evidence to support association

5.3 Modalities of Ovarian Reserve Testing

Broadly speaking, in conjunction with a proper history and physical exam, there are at least three common modalities of ORTs: imaging, biomarker testing, and ovarian response itself (Table 5.2).

5.3.1 Imaging

Ultrasonography is the imaging modality of choice for testing ovarian reserve generally via a transvaginal ultrasound probe which can provide ovarian volume measurements or antral follicle counts (AFC). AFC is the more commonly used metric in the literature and identifies follicles

generally from 2 to 10 mm in diameter [30]. Although AFC is most frequently obtained manually counting follicle diameter, there are efforts to automate the processing of the images to provide count and volume measurements with the thought there would be less user-dependent variability [31, 32]. Ovarian volume has also been considered as a potential ORT, but studies demonstrate it not to be as predictive of ovarian response as AFC [5, 30, 33]

5.3.2 Biomarkers

Biomarker testing primary involves biochemical evaluation of the hypothalamic pituitary ovarian (HPO). A frequently used biomarker historically and currently is follicle stimulating hormone (FSH) which is secreted by the pituitary and is well known to begin to rise early in the menstrual cycle to stimulate follicles to mature and become candidates for ovulation [34]. Excess FSH secretion and follicle stimulation is prevented through subsequent FSH suppression by rising levels of estradiol from oocytes, as well as by the glycoprotein hormone, inhibin B, which is produced by granulosa cells of pre-antral and antral follicles [35]. FSH secretion may vary widely from cycle to cycle (perhaps warranting the nickname "Fluctuating Severely Hormone"), with the prognostic value of the test being most accurate with the highest values [36, 37]. This fluctuation creates the problem that FSH may often be falsely reassuring regarding the status of ovarian reserve [38]. Antimullerian hormone (AMH) is also a glycoprotein secreted by granulosa cells like inhibin B but from early stage follicles and acts to inhibit FSH effects on the follicle [39]. AMH is different from FSH and inhibin b in that levels during the menstrual cycle remain fairly constant when averaged across a population [40–44]. However, it should be emphasized that within individuals, there can be significant changes in AMH levels within a cycle [45]. While AMH variability is clinically significant (perhaps also deserving a nickname, "Also Meandering Hormone") it shows less variability than most other ORTs when remeasured. Lastly, AMH has been shown at a population level to decline gradually in an almost linear fashion [46–49], while FSH is known to remain relatively constant or rise slowly until a rapid rise is observed in the perimenopausal stage [19]. An important area of research is to determine within individuals what patterns of AMH decline exist which underlie the gradual age-dependent decline in average AMH values observed at a population level. It also is possible that at some point in the future, genetic markers such as FMR1 will also be tested more routinely to help predict whether a woman is at risk for development of premature depletion of oocyte supply [50, 51].

5.3.3 Ovarian Response

Incorporation of the patient response to the diagnostic process can be assessed with a mixture of medication and multiple biomarker measurements, referred to as dynamic or provocative testing. In addition, the actual outcome of an ART cycle itself has been reported to predict future response in certain patient populations. Commonly cited dynamic tests include the clomiphene citrate challenge test (CCCT) which measures serum FSH just prior and after 5 days of clomiphene treatment beginning on cycle day 5; the exogenous FSH ovarian reserve test (EFORT) which measures serum FSH and/or inhibin B just prior to administration FSH on cycle day 3, then measured again 24 h later [1, 5, 52–54]. Attempting to incorporate patient response into the diagnostic assessment is expensive and logistically difficult which likely has decreased the prevalence of the use of this modality. However, ultimately, the number of high quality oocytes retrieved in COS may be considered one of the major clinical outcomes of interest and closest surrogate for quantitative aspects of ovarian reserve. Thus, the patient's response to COS itself serves as a helpful modality to assess ovarian reserve [55, 56].

5.3.4 Multivariate Approaches

As more ORTs become available and more patient subphenotypes are defined, the clinician is faced with an increasing number of variables. This presents the challenges of answering which tests

are most predictive of the outcome of interest, are several tests better than one test, and how should the tests be weighted? The reality is each clinician uses a multivariate approach when making daily decisions, often referred to as the "art of medicine." The clinician must intuitively weight dozens of variables contained in the past medical history, age, and physical exam with the ORT results but without clear data about how many of these inputs change accuracy. Attempts are now being made to potentially improve the performance of ORTs by combining them mathematically in algorithms to allow optimized weighting and produce clinically usable information [31, 57–61]. The same issues that prevent consensus with single ORT use are magnified with use of index scores and multivariate approaches—which makes it even more difficult to compare studies. Currently, the gains shown by published studies are modest at best for use of ORTs and age at predicting COS response and success of ART treatments and have conflicting conclusions. Meta analyses that seek to combine data from multiple centers and laboratories can be problematic given the heterogeneity of the testing methods, patients, and treatment protocols and it is not surprising that they obtain results that show poor associations [62–64]. Yet, if multivariate models are used to synthesize consistent ORT methodology, patient populations, and treatments, it is quite possible that the information obtained from combining biomarkers, imaging techniques, and genetic variants, will be more informative and easier to apply clinically.

5.4 Current Clinical Applications of Ovarian Reserve Tests

Descriptions regarding the current clinical applications of ORTs (Table 5.2) are provided below but there are certain caveats that apply almost uniformly to these applications:

- First, the wide variety of definitions used for patient populations, exposures, ORT selection, and methodology, prevents any actual cut points from being generally recommended without first defining the aforementioned variables precisely.
- Secondly, ORT values exist on a continuum and can fluctuate within individuals due to inherent biological variability, such that single measurements can be misleading with frequencies that depend upon the ORT and patient population. Thus, cut points for ORTs, which are useful to compare assays or establish clinical algorithms, should be used cautiously and the reliance on one ORT modality should be avoided for definitive management decisions.

The consequence of these caveats is that practical approaches may require more effort by the clinician when initially establishing a clinical strategy to navigating the use of ORTs including (a) gaining an understand from where cut points and value ranges were derived for a chosen ORT source and (b) if that relates appropriately to the clinical outcomes and patient population being managed.

5.4.1 ORTs for Predicting Response To Controlled Ovarian Stimulation

Although additional applications of ORTs are developing and in clinical practice, identifying low and high responders to COS may be the most well-established use. The term "low" rather than "poor" and "high" rather than "good" is selected here to emphasize and focus on the quantitative aspect of response to COS separately from oocyte quality and ART cycle success.

5.4.2 Low Responders

The literature can be confusing as most of the ORTs have studies demonstrating cut points which can yield sensitivities and/or specificities above 80–90 %. There now have been a number of studies that have compared the performance characteristics of most ORTs together, including basal FSH, inhibin B, estradiol, AMH, and AFC. AMH and AFC perform fairly consistently with greater overall correlation to low response than age or other single ORTs, which, given the heterogeneity of study designs, attests to their strong correlation to response to COS [1, 3, 6, 21–23, 31, 54, 65]. Although some studies have tried to

determine which performs better, AFC or AMH, results have shown fairly similar performances when both ORTs are performed well although some may believe AFC to be slightly better than AMH when in the hands of experienced clinicians [66, 67]. It should be noted, however, that none of the ORTs have demonstrated, through multiple publications from several groups, sufficient sensitivity or specificity to predict with certainty the outcome in ART, even for oocyte quantity.

Some studies have shown basal serum FSH to have clinically helpful specificity for poor response [36, 68], with one study showing of up to 100 % specificity but only when a high cut point for normal is utilized and with sensitivity too low to be used alone as an ORT [68]. Basal Inhibin B, initially showed promise as an ORT in studies using the inhibin B system from Serotec, LTD [69, 70]. However, subsequent studies [1, 6] failed to reproduce similar accuracy for inhibin B, commensurate, interestingly, with the lack of availability of the Serotec platform. Dynamic or provocative tests such as CCCT and EFORT (using both FSH and inhibin B) [2, 71–73] have consistently shown clinically useful sensitivity and/or specificity often superior to other single ORTs. However, the requirement for two measurements and medication has likely led to minimal use, especially when evidence exists that a single measurement of a single ORT may have sufficiently similar accuracy [54].

5.4.3 High Responders

Certain ORTs consistently demonstrate the significant ability to predict, independently of age, which women will likely be high responders to COS which has important benefits to reduce complications of excessive response (e.g., OHSS and cycle cancelation) and also to reduce consumption of gonadoptropins. There are now numerous studies demonstrating clinical utility of ORTs with respect to a wide variety of definitions of excessive response including high estradiol levels, withdrawal of stimulation (“coasting”), cycle cancelation, high number of oocytes retrieved, and more severe conditions associated with OHSS such as accumulation of ascites and hospitalization [8, 54, 62, 74–76]. For example, in a study of 110 patients with excessive response defined as greater than 20 oocytes retrieved, investigators could demonstrate that an AFC cut point could select 11 % of patients and identify hyper-response with 50 % and 96 % sensitivity and specificity, respectively [54]. Using moderate and severe OHSS as a clinical outcome, in a study of 262 patients, an AMH cut point which identified 25 % of the patients also performed with 91 % sensitivity and 81 % specificity, respectively for OHSS [8]. However, despite the variation in the definition of excessive response outcome and also variation with cut point selection, AFC and AMH showed across multiple studies clinical helpful performance characteristics and frequently performed better than most other ORTs for both sensitivity and specificity. As both AMH and AFC measurements exist along a continuum, for practical implementation, one must chose the definition of excessive response and identify internal thresholds for management changes.

5.4.4 Oocyte Quality, Live Birth Rate in ART

With respect to ART treatments, the studies performed to date have not demonstrated with sufficient consistency or robust predictive power a clinically helpful relationship between ORT results and oocyte quality or pregnancy success that is widely applicable with specific cut points [19]. That said there have been studies which demonstrate remarkable results in specific circumstances that could dramatically help guide care. For example, in a study of serum basal FSH measurements in over 8,000 cycles from one center with a single FSH measurement source, FSH thresholds could make clinically helpful, age group specific, robust predictions of chances of live delivery per ART cycle start along a continuum of values [68]. Values above certain thresholds demonstrated 100 % specificity for failed cycles although those thresholds only identified about 1 in 30 women tested above 40 years of age and 1 in 324 women tested under age 35. However, other differently structured studies arrive at dif-

ferent conclusions such as FSH being valuable predicting live birth only in certain age groups [77] versus no ability to predict live birth better than age alone as concluded by a recent meta-analysis which used 28 databases to aggregate data from 5,705 IVF patients and multiple FSH diagnostic platforms [64]. A number of studies indicate that AMH or AFC levels do not predict treatment success [21, 78]. This conflicts with the findings other published findings [60, 76, 79] including a recent study externally validated an AMH-based live birth prediction model to, independent of age, predict live birth in 822 patients with statistical significance, although the confidence intervals were wider than some may view as clinically helpful [59].

The lack of consensus and conflicting medical literature is not surprising given the multifactorial nature of embryo development into a healthy baby. However, the heterogeneity of study designs, an inability to control for confounding variables, and insufficiently robust biological association of ORTs to live birth rate, presents serious hurdles to overcome in the quest for consensus. Thus, applications of ORTs in predicting live birth currently must remain a user-defined, site-specific approach. Future studies that examine the prognostic value of AMH, AFC, or other tests on cumulative live birth rate or total reproductive potential as described above are needed. It is quite plausible that any measure that predicts oocyte number of retrieval may be a better predictor of the success of fresh and frozen embryo transfer combined, than it would be of fresh cycles only because more embryos are likely to be frozen if a greater number of oocytes are retrieved.

5.4.5 Overall Fertility and Recurrent Pregnancy Loss

Clinical justification for ORT use in the general population to assess fertility is beginning to appear. Evidence is mounting that infertility is associated with lower ORT values as demonstrated by lower AFC in 881 infertile women without PCOS compared to 771 women without the diagnosis of infertility [16]. In another prospective study of 100 general population women attempting to conceive, early follicular phase AMH was shown to predict fertility rates [17]. Thus, it appears promising that ORT results will play a future role in fertility assessment of the general population.

Data on miscarriage and ORTs are scant and primarily derive from patients receiving ART treatment. One retrospective study showed no association with highest serum basal FSH and fetal aneuploidy [80] in 177 spontaneous miscarriages associated with 70 euploid and 107 aneuploid offspring. No association with AFC, FSH, and CCCT was demonstrated prospectively comparing values in 77 women with pregnancy loss versus 233 with ongoing pregnancy [52]. However, AFC was shown to be predictive of only first trimester loss in 67 patients with miscarriage compared to 247 controls with ongoing pregnancy, although the overall association was weak with an ROC curve AUC of 0.588 [81]. Recently, in a study of women undergoing aneuploidy screening of embryos followed by IVF of 279 women, those with reassuring FSH and AMH values generated lower rate of all aneuploid blastocysts compared to 93 women with concerning FSH and/or AMH (35 % vs. 14%, $P<0.001$) [82]. It was further noted that when both FSH and AMH were concerning, the highest percentage of aneuploid blastocysts was observed (77 %) compared to only one being concerning (58.5 %, 58.8 %) and both reassuring (51.7 %). Thus, it appears that ORTs may be useful in predicting increased risk of miscarriage.

5.4.6 PCOS, POI, and Menopause

As research has advanced and ORTs such as AFC and AMH have become more widely used, helpful clinical information for patients can be applied to help identify, diagnose, and manage other diseases and processes not strictly related to attempts to have a child.

AMH is now also being proposed by some as an alternative criterion to diagnose women with PCOS or to identify women at high risk for PCOS [83]. One recent study, which included by 66 women without

PCOS or polycystic ovaries and 62 confirmed PCOS by hyperandrogenism and oligomenorrhea, identified an optimized AMH cut point demonstrating 92 % sensitivity and 97 % specificity for PCOS [84]. However, the use of AMH in this context remains controversial and has not been adopted in official criteria for PCOS diagnosis.

Perhaps the most exciting developments relate to early detection of POI and long-term prediction of the menopausal transition and menopause. As AMH and AFC levels, at a population level, demonstrate a gradual almost linear decline [46–49], these ORTs have applications in both early detection of POI prior to symptoms and long-term prediction of menopause onset. Earlier identification of women at risk of POI may help them avoid the most severe consequences of this disease such as missing the opportunity to have children with their own eggs as well as other complications associated with early menopause such as bone loss and increased cardiovascular events [14].

Although AMH, AFC, FSH, and Inhibin B have all been published as being able to add significantly more predictive power to prediction of menopause than age alone, AMH and AFC appear to show the better performance characteristics [11–13, 85]. Furthermore, it may be rates of change are more predictive than single measurements [85, 86]. As increasing amounts of individualized longitudinal data are becoming available, the confidence intervals around age of the predicted last menstrual period are becoming narrower [87]. Subphenotypes may be further defined that can increase predictive information, such as genetic interactions with ovarian reserve. For example, one study of 240 women indicated that FMR1 repeat length was associated with a 54 % difference in AMH level [50]. Another recent study identified several genetic markers in 450 women that were associated with ovarian follicle number and menopause [88]. At this juncture, the published literature on menopause prediction appears sufficiently consistent such that, if a women has an AMH or AFC value very low for her age using a well-calibrated testing source, it would be questionable not to alert her at least about the increased possibility of earlier than average menopause. This knowledge can allow a woman to proactively address her desired plan for future childbearing. In addition, a woman with ORT results substantially low for her age can proactively address the risk of long-term medical issues such as osteoporosis, cardiovascular disease, and certain forms of cancer which are more prevalent in women with early menopause [14].

With the availability of clinically validated egg preservation technologies, there is now the ability to dramatically increase the length of time a woman has to have a child with her own eggs [89]. This significant advancement has clear immediate application to preserve eggs, for example, prior to receiving ovarian toxic treatments such as chemotherapy [90]. However, the combination of egg preservation and the developing predictive power of ORTs, presents society with the double-edged sword of providing a safety net for possible future ovarian reserve-related infertility, but the risk of encouraging women to delay natural attempts at conception.

5.4.7 Exogenous Hormone Use

Influence on AMH levels by exogenous hormones has been clearly demonstrated [91]. While some publications suggest that oral contraceptive pills (OCPs) do not affect AMH or AFC levels [92], it now is becoming clear that OCPs such as monophasic estrogens can lower AMH and AFC levels [93, 94]. In one study of 25 women on OCPs for more than 3 months significant improvement in AMH and AFC parameters were observed after the second menstrual cycle without OCPs [95]. This was confirmed in a complementary study with 44 women off OCPs for at least 3 months who showed an average reduction of approximately 50 % in AMH by week 9 of OCP use [96]. This indicates that if a woman has a concerning AMH or AFC while on an estrogen OCP, it may be helpful to retest after stopping the OCP use for two cycles if the retesting would change management. However, if the AMH level is reassuring while on estrogen OCPs, the above recently published studies indicate it will likely remain reassuring off OCPs. While there may be logical ways to extract clinically helpful information in certain scenarios with patients taking

OCPs, careful attention should be paid to the use of exogenous hormones when interpreting ORTs.

5.5 Current Challenges

5.5.1 Biology

One of the biggest barriers for current ORTs in achieving the desired narrowness of confidence intervals for predicting clinical outcomes is the inherent biological flux associated with biomarkers of the HPO axis. If ORT results can fluctuate in clinically significant amounts with some frequency, there is an intrinsic limit to the accuracy of the test regardless of study design, sample size, and uniformity of patient population. It has long been recognized that FSH levels fluctuate dramatically between from cycle to cycle [36, 37]. The recently more popular ORTs, AFC and AMH, receive much focus in part because the average value in the population does not show the same dramatic dependence on the stage of the menstrual cycle as FSH, inhibin B, LH, or estradiol [44, 97]. While this has important logistical benefits by not requiring measurement at a particular time of the menstrual cycle, especially in those women who do not regularly menstruate or have had a hysterectomy, this does not address the larger issue of values being clinically significantly different in the same individual when retested even within the same menstrual cycle. For example, Sowers et al. measured AMH every day of the menstrual cycle, demonstrating a consistent AMH average throughout the menstrual cycle in five groups of five women with similar AMH values [44]. However, closer examination of the data points showed two of five women with similar average AMH values having daily values of approximately 0.6 and 0.75 ng/ml for half the cycle and nearly 2 ng/ml for the other half of the menstrual cycle. This finding was recently observed again in a population of 44 women retested within a menstrual cycle [45].

The other major biologic barrier for ORTs to assess accurately the ability of a woman's oocytes to produce a healthy baby, is that from fertilization onward, numerous other confounding variables are required in the process. A successful pregnancy depends upon many factors such as a sufficiently healthy sperm and a receptive endometrium. This presents a significant challenge both in the current ability to diagnostically assess these variables accurately and separately, and, statistically, in the number of patients needed to appropriately power studies that would seek to perform the extensive subset analysis required.

5.5.2 Standardization

While the biology of the human reproductive system is difficult to control, the fertility field is challenged with lack of consistency in almost every aspect of ORT study design to the point that the latest American Society for Reproductive Medicine practice guideline concluded that there is no consensus as to the definition of ovarian reserve and the evidence for the tests which measure it is at best "fair" [19]. Substantial variation can be seen in study population phenotypes, treatment regimes, clinical outcomes assessed, choice of ORT(s), and method of analysis, and use of cut points.

When it comes to performance of the ORTs, dramatic differences can exist in the reported value and clinical performance for the same sample depending upon the diagnostic platform chosen (Fig. 5.1). The best example perhaps of this is the history of inhibin B which showed clinically useful performance with the Serotec kit [69, 70] and not with the DSL kit that replaced it, leading to the likely unrepairable clinical distrust of this biomarker [1, 6]. One misconception is that automation and FDA clearance resolve issues with consensus. While FDA clearance and automation improved the assay performance and ease of measurement for serum FSH, this has not led to establishment of consensus regarding FSH testing despite over 20 years of publications regarding its use [19]. Differences in diagnostic platforms are not clear on reports provided to clinicians frequently. These differences can be substantial as in the recent College of American Pathology Surveys 2011 Y-B Ligand publication demonstrated that 434 laboratories produced an

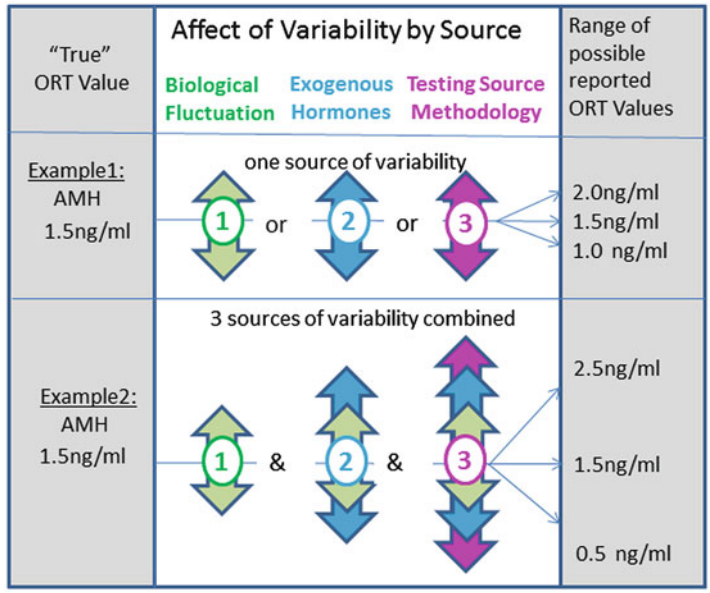

Fig. 5.1 Effect on reported ORT value by three different sources of variability. When retesting the same patient with an ORT, a minority, but significant fraction of the time one value is clinically different from a patient's "true" or most representative value. At least three factors can affect this. (1) Biological flux of ORTs can be substantial. (2) Exposures to medications such as oral contraceptive are now known to affect results of ORTs such as AMH and AFC. (3) Although testing methods may have minimal variability within a chosen source, the between source assay differences may be substantial. The affect of any single source of variability can be clinically significant (example 1) and even more so if multiple sources of variability are present and combine I the same direction (example 2)

acceptable mean FSH value of 34 IU/L while, with the same reference sample, 151 other laboratories produced an acceptable mean value of 19 IU/L, the difference being the FSH analyzer platform.

Unfortunately, for the two ORTs currently receiving the most attention, AFC and AMH, reference standards don't even exist. AFC is a highly user-dependent modality and, despite attempts at international standardization, there remains some inconsistency in the size of follicle to include in the AFC with obesity further complicating interpretation or rending it impossible [30]. The measurement of AMH has undergone three kit changes (Immunotech, DSL "GenI," Beckman/DSL "GenII") in the past 3 years, with a new one arriving on the market shortly along with automated platforms and blood spot tests on the way [98–100]. Although the clinical correlations observed with different AMH kits are consistent, different AMH kits often have inconsistent conversion equations published between the others. This makes extrapolation of results from one kit to another risky to interpret a clinical report for a patient without conducting careful validation experiments. It is also very important for clinicians to be aware that values in the literature may have been performed using a different assay and thus may not be readily applicable to the results of their patients. Additionally, the use of the same AMH kit can produce dramatically different values depending on a variety of factors including the treatment of sample and the laboratory methodology. Furthermore, as previously discussed, the influence of exogenous medications such as OCPs were once considered of no consequence now are recognized as significantly affecting ORT results. However, most importantly clinical value ranges, which determine the treatment, are frequently set by the laboratory based upon CLIA requirements to establish a general mean and distribution in a general population and not upon the

Table 5.3 Practical steps to optimize the use of ORTs

6 Steps to optimize ORT use	
Recognize lack of consensus for ORT use	Recognize consensus does not currently exist regarding ORT interpretation and that utility but depends upon your chosen ORT, clinical parameters, patient population, and clinical purpose
Identify at least two ORTs	Choose at least two ORTs from preferably two different modalities if available (e.g., AFC and AMH). Minimizes impact of variability of an individual ORT fluctuation
Establish how ORT clinical value ranges were generated	Identify a consistent source of ORTs if possible and understand how value ranges relate to clinical outcomes being manages. Avoid applying thresholds used in publications which utilize ORT sources with no link to your current source. Ideally establish internal value ranges for any source of ORT
Verify no changes in calibration on a regular basis	Unfortunately, there are frequent changes in assay materials, lab methodologies, treatment modalities that can change the interpretation of results. Verify if practical once every 6 months to a year with the laboratory director of any consistently used sources of ORTs
Be aware estrogen-based OCPs may lower AMH and AFC	Recent data suggest estrogen-based OCPs can lower AMH and AFC results. However, if a patient already has reassuring values on OCPs, it is likely they would remain reassuring off OCPs. If consider retesting on the second natural cycle off OCPs if a different ORT result would change management
Avoid definitive predictions based	ORT information should refine, not define clinical management

clinical outcomes being managed by the test. For example, a "normal range" for AMH can be 0 ng/ml to 6.9 ng/ml which spans the gamut of ovarian failure (depleted ovarian reserve) to high risk for OHSS or PCOS (high ovarian reserve).

The above challenges can unfortunately be additive and pose a significant risk of clinically miscategorizing a patient if careful steps are not taken to avoid this (Fig 5.1). Fortunately, there are practical ways to minimize the chance of misguiding care with ORT use.

5.6 Practically Optimizing the Use Of ORTs

5.6.1 It's The Approach, Not Just the Test

The pattern that consistently emerges from literature assessing ORTs is that performance and utility depend upon the user's decisions regarding patient populations, treatments, ORT selection, and methodology. Furthermore, the value of a particular ORT's PPV and NPV depends upon prevalence of the clinical outcome in the intended use population, which can vary dramatically, for example, with diminished ovarian reserve in an oocyte donor screening program as compared to counseling a woman about IVF using her own oocytes. Thus, minimizing the risk of misinterpretation of ORT values requires a methodical approach, which may involve some initial effort to establish (Table 5.3). One approach is described below:

- The first recommended practical step is to recognize that consensus does not currently exist regarding ORT interpretation and utility and expend the effort necessary to establish one's desired approach.
- Second, the fluctuation of ORT results and possible sources of error makes important utilizing at least two different ORTs when evaluating a patient. Frequently, this is possible as other ORTs, such as FSH and estradiol, have other utilities in the initial assessment of a patient, and therefore to combine this with AFC and/or AMH is logistically reasonable. The use of different modalities such as imaging and serum testing has the added benefit of it being less likely to have an error, such as improper specimen handling, affect both modalities.

- Third, it would be ideal, but frequently not possible, to establish a consistent source of ORTs and obtain an understanding of how the clinical value ranges are determined. The ideal scenario is that each practitioner ultimately clinically calibrates his/her ORTs against his/her own outcome data, but this is often times not feasible. Practically speaking, as it is not possible to track down the source of every outside laboratory result, judicious use of retesting at a familiar source should be considered if retesting could significantly change clinical management.
- Although perhaps an unpleasant truth, the materials and methods change, not infrequently, for ORTs and vigilance with respect to the affect this change would have on interpretation is important. If one uses regularly one or two sources for ORT results it would not be unreasonable to perform a brief inquiry of the laboratory director once or twice per year as to if there were any changes with a chosen source of ORT that could affect value ranges.
- Fifth, as many women use OCPs and it is difficult at times to stop taking them, a practical method for tests such as AMH or AFC is to obtain the values and if reassuring consider it sufficient to use this value as recent data indicates it is likely that the ORT result remain reassuring if not more so off estrogen OCPs. If AMH and AFC are concerning while on an estrogen-based OCP, one can consider then retesting off OCPs if management decisions would change.
- Sixth, overall, one should be very cautious and avoid, if possible, counseling a patient solely based upon ORT values since the certainty of outcome for these tests is not definitive. Ultimately, it is advisable to use ORTs to influence rather than direct clinical management.

5.6.2 Clinical Example

Given the especially ambiguous nature of ORT results and lack of consensus, a short case sce-

Case 1

A healthy 28-year-old female with no prior attempts at conception is considering attending medical school and presents to fertility specialist, referred by general practitioner with an AMH value of 2.0 ng/ml by an outside laboratory with normal range reported as 0–6.9 ng/ml.

Patient: "Will I still be able to have children in 8 years after I finish medical school and residency?"

Clinician: "With no family or medical history concerning for early loss of fertility, it would be wise to recheck this lab value before drawing any conclusions. In the meantime, let's obtain an antral follicle count today by ultrasound"

AFC shows a total of ten follicles between 2 and 10 mm. Rechecking of the AMH at a different laboratory regularly used by the clinician with well-established value ranges returns value of 0.6 ng/ml which fell into a range that was consistent with the patient already being at high risk for poor egg supply. Discussion at next visit:

Clinician: "Rechecking your AMH shows you have a value that is low for your age and that you already are at risk for low egg supply. There are now several studies from several sources that show women with low AMHs are more likely to go into menopause sooner than women with high AMHs of the same age. While we can't give you any specific prediction about your fertility window, you are at likely at higher risk than the average to have menopause earlier and thereby have a shorter fertility window. If having children right now is not want you want or are able to do, you may want to consider egg cryopreservation. While long term follow up data isn't yet available we are cautiously optimistic about there not being significant difference between babies born through natural conception versus

(continued)

Case 1 (continued)

babies born through IVF using preserved oocytes."

Patient: "Why was my AMH lab value so different when you retested it?"

Clinician: "It could be natural fluctuation or differences in the methods of measurement. Let's revaluate in the next several months and also try to figure out what you would like to do about planning your future family building goals."

nario is presented with possible responses to better illustrate use of the recommendations. This scenario is not intended to represent consensus views or incontrovertible information.

5.7 Conclusion

Research over the past 10 years has demonstrated a wide variety of clinical utility for ORTs such as improving COS management, risk stratification for ART treatment success, identification of women at risk for infertility, more sensitive detection of diminished ovarian reserve, prediction of time until menopause, and adjunctive use to identify and/or diagnose PCOS. The ORTs, AMH and AFC, have each emerged as the two most predictive individual ORTs for responsiveness to COS for retrieval of oocytes as well as sensitive identifiers of diminished oocyte supply, proximity to menopause, and likelihood of PCOS. Many of these research findings are currently applied with clinical benefit.

While the potential advantages of ORT use in clinical medicine is clear, with the biological fluctuations in ORT results, the complexity of fertility assessment, and lack of standardization, consensus is not possible regarding most of the above utilities, and the risk of misguiding clinical care using an ORT result is high if appropriate steps are not taken by clinicians. This risk can be minimized by (1) recognizing that performance of an ORT is specific to the source of ORT and the clinical environment in which it is applied, (2) identifying at least two different ORTs for use, (3) use a consistent source of ORT results where possible with an understanding of how the values relate to the clinical outcomes being managed, (4) inquiring periodically about assay change at a chosen ORT source which could change interpretation, (5) avoiding use cut points from publications without understanding how they apply to your source of ORT, (6) paying attention to exogenous hormone use, and (7) avoiding the use ORTs alone to make clinical decisions. This approach likely will reduce the risk of misinterpretation of results while simultaneously harnessing the information ORTs can provide to improve clinical care.

References

1. Broekmans FJ, Kwee J, Hendriks DJ, et al. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update. 2006;12(6):685–718.
2. Dzik A, Lambert-Messerlian G, Izzo VM, et al. Inhibin B response to EFORT is associated with the outcome of oocyte retrieval in the subsequent in vitro fertilization cycle. Fertil Steril. 2000;74(6):1114–7.
3. Gnoth C, Schuring AN, Friol K, et al. Relevance of anti-Mullerian hormone measurement in a routine IVF program. Hum Reprod. 2008;23(6):1359–65.
4. Jee BC, Ku SY, Suh CS, et al. Serum anti-Mullerian hormone and inhibin B levels at ovulation triggering day can predict the number of immature oocytes retrieved in in vitro fertilization cycles. J Korean Med Sci. 2008;23(4):657–61.
5. Kwee J, Elting ME, Schats R, et al. Ovarian volume and antral follicle count for the prediction of low and hyper responders with in vitro fertilization. Reprod Biol Endocrinol. 2007;5:9.
6. Riggs RM, Duran EH, Baker MW, et al. Assessment of ovarian reserve with anti-Mullerian hormone: a comparison of the predictive value of anti-Mullerian hormone, follicle-stimulating hormone, inhibin B, and age. Am J Obstet Gynecol. 2008;199(2):202.e1–8.
7. van Tilborg TC, Eijkemans MJ, Laven JS, Koks CA, de Bruin JP, Scheffer GJ, et al. The OPTIMIST study: optimisation of cost effectiveness through individualised FSH stimulation dosages for IVF treatment. A randomised controlled trial. BMC Womens Health. 2012;12:29.
8. Lee TH, Liu CH, Huang CC, et al. Serum anti-Mullerian hormone and estradiol levels as predictors of ovarian hyperstimulation syndrome in assisted

reproduction technology cycles. Hum Reprod. 2008;23(1):160–7.
9. Yates AP, Rustamov O, Roberts SA, et al. Anti-Mullerian hormone-tailored stimulation protocols improve outcomes whilst reducing adverse effects and costs of IVF. Hum Reprod. 2011;26(9):2353–62.
10. Freeman EW, Sammel MD, Lin H, et al. Anti-Mullerian hormone as a predictor of time to menopause in late reproductive age women. J Clin Endocrinol Metab. 2012;97(5):1673–80.
11. Sowers MR, Eyvazzadeh AD, McConnell D, et al. Anti-Mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. J Clin Endocrinol Metab. 2008;93(9):3478–83.
12. Tehrani FR, Shakeri N, Solaymani-Dodaran M, et al. Predicting age at menopause from serum antimullerian hormone concentration. Menopause. 2011; 18(7):766–70.
13. Tehrani FR, Solaymani-Dodaran M, Azizi F. A single test of antimullerian hormone in late reproductive-aged women is a good predictor of menopause. Menopause. 2009;16(4):797–802.
14. Nelson LM. Clinical practice. Primary ovarian insufficiency. N Engl J Med. 2009;360(6):606–14.
15. Practice Committee of the American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: a committee opinion. Fertil Steril. 2012;98(6):1407–15.
16. Rosen MP, Johnstone E, Addauan-Andersen C, et al. A lower antral follicle count is associated with infertility. Fertil Steril. 2011;95(6):1950–4. 4 e1.
17. Steiner AZ, Herring AH, Kesner JS, et al. Antimullerian hormone as a predictor of natural fecundability in women aged 30–42 years. Obstet Gynecol. 2011;117(4):798–804.
18. White YA, Woods DC, Takai Y, et al. Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. Nat Med. 2012; 18(3):413–21.
19. Harlow SD, Gass M, Hall JE, et al. Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. Fertil Steril. 2012;97(4): 843–51.
20. Hansen KR, Knowlton NS, Thyer AC, et al. A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. Hum Reprod. 2008;23(3):699–708.
21. Riggs R, Kimble T, Oehninger S, et al. Anti-Mullerian hormone serum levels predict response to controlled ovarian hyperstimulation but not embryo quality or pregnancy outcome in oocyte donation. Fertil Steril. 2011;95(1):410–2.
22. Hazout A, Bouchard P, Seifer DB, et al. Serum antimullerian hormone/mullerian-inhibiting substance appears to be a more discriminatory marker of assisted reproductive technology outcome than follicle-stimulating hormone, inhibin B, or estradiol. Fertil Steril. 2004;82(5):1323–9.
23. Knauff EA, Eijkemans MJ, Lambalk CB, et al. Anti-Mullerian hormone, inhibin B, and antral follicle count in young women with ovarian failure. J Clin Endocrinol Metab. 2009;94(3):786–92.
24. Kunt C, Ozaksit G, Keskin Kurt R, et al. Anti-Mullerian hormone is a better marker than inhibin B, follicle stimulating hormone, estradiol or antral follicle count in predicting the outcome of in vitro fertilization. Arch Gynecol Obstet. 2011;283(6):1415–21.
25. Forman EJ, Hong KH, Treff NR, et al. Comprehensive chromosome screening and embryo selection: moving toward single euploid blastocyst transfer. Semin Reprod Med. 2012;30(3):236–42.
26. Forman EJ, Tao X, Ferry KM, et al. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. Hum Reprod. 2012; 27(4):1217–22.
27. Treff NR, Su J, Kasabwala N, et al. Robust embryo identification using first polar body single nucleotide polymorphism microarray-based DNA fingerprinting. Fertil Steril. 2010;93(7):2453–5.
28. Luke B, Brown MB, Wantman E, et al. Cumulative birth rates with linked assisted reproductive technology cycles. N Engl J Med. 2012;366(26):2483–91.
29. Stern JE, Hickman TN, Kinzer D, et al. Can the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) be used to accurately report clinic total reproductive potential (TRP)? Fertil Steril. 2012;97(4):886–9.
30. Broekmans FJ, de Ziegler D, Howles CM, et al. The antral follicle count: practical recommendations for better standardization. Fertil Steril. 2010;94(3): 1044–51.
31. Nelson SM. Biomarkers of ovarian response: current and future applications. Fertil Steril. 2013 Jan 8.
32. Jayaprakasan K, Campbell B, Hopkisson J, et al. A prospective, comparative analysis of anti-Mullerian hormone, inhibin-B, and three-dimensional ultrasound determinants of ovarian reserve in the prediction of poor response to controlled ovarian stimulation. Fertil Steril. 2010;93(3):855–64.
33. Johnson NP, Bagrie EM, Coomarasamy A, et al. Ovarian reserve tests for predicting fertility outcomes for assisted reproductive technology: the International Systematic Collaboration of Ovarian Reserve Evaluation protocol for a systematic review of ovarian reserve test accuracy. BJOG. 2006; 113(12):1472–80.
34. Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. Endocr Rev. 2009;30(5):465–93.
35. Klein NA, Houmard BS, Hansen KR, et al. Age-related analysis of inhibin A, inhibin B, and activin a relative to the intercycle monotropic follicle-stimulating hormone rise in normal ovulatory women. J Clin Endocrinol Metab. 2004;89(6):2977–81.
36. Scott RT, Toner JP, Muasher SJ, et al. Follicle-stimulating hormone levels on cycle day 3 are pre-

dictive of in vitro fertilization outcome. Fertil Steril. 1989;51(4):651–4.
37. Sharif K, Elgendy M, Lashen H, et al. Age and basal follicle stimulating hormone as predictors of in vitro fertilisation outcome. Br J Obstet Gynaecol. 1998;105(1):107–12.
38. Leader B, Hegde A, Baca Q, et al. High frequency of discordance between antimullerian hormone and follicle-stimulating hormone levels in serum from estradiol-confirmed days 2 to 4 of the menstrual cycle from 5,354 women in U.S. fertility centers. Fertil Steril. 2012;98(4):1037–42.
39. Nilsson EE, Schindler R, Savenkova MI, Skinner MK. Inhibitory Actions of Anti-Mullerian Hormone (AMH) on Ovarian Primordial Follicle Assembly. PLoS One. 2011;6(5):e20087.
40. Hehenkamp WJ, Looman CW, Themmen AP, et al. Anti-Mullerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. J Clin Endocrinol Metab. 2006;91(10):4057–63.
41. La Marca A, Giulini S, Tirelli A, et al. Anti-Mullerian hormone measurement on any day of the menstrual cycle strongly predicts ovarian response in assisted reproductive technology. Hum Reprod. 2007;22(3):766–71.
42. La Marca A, Sighinolfi G, Giulini S, et al. Normal serum concentrations of anti-Mullerian hormone in women with regular menstrual cycles. Reprod Biomed Online. 2010;21(4):463–9.
43. La Marca A, Stabile G, Artenisio AC, et al. Serum anti-Mullerian hormone throughout the human menstrual cycle. Hum Reprod. 2006;21(12):3103–7.
44. Sowers M, McConnell D, Gast K, et al. Anti-Mullerian hormone and inhibin B variability during normal menstrual cycles. Fertil Steril. 2010;94(4):1482–6.
45. Overbeek A, Broekmans FJ, Hehenkamp WJ, et al. Intra-cycle fluctuations of anti-Mullerian hormone in normal women with a regular cycle: a re-analysis. Reprod Biomed Online. 2012;24(6):664–9.
46. Seifer DB, Baker VL, Leader B. Age-specific serum anti-Mullerian hormone values for 17,120 women presenting to fertility centers within the United States. Fertil Steril. 2011;95(2):747–50.
47. Almog B, Shehata F, Suissa S, et al. Age-related normograms of serum antimullerian hormone levels in a population of infertile women: a multicenter study. Fertil Steril. 2011;95(7):2359–63. 63 e1.
48. Nelson SM, Messow MC, McConnachie A, et al. External validation of nomogram for the decline in serum anti-Mullerian hormone in women: a population study of 15,834 infertility patients. Reprod Biomed Online. 2011;23(2):204–6.
49. Nelson SM, Messow MC, Wallace AM, et al. Nomogram for the decline in serum antimullerian hormone: a population study of 9,601 infertility patients. Fertil Steril. 2011;95(2):736–41. e1–3.
50. Spath MA, Feuth TB, Allen EG, et al. Intra-individual stability over time of standardized anti-Mullerian hormone in FMR1 premutation carriers. Hum Reprod. 2011;26(8):2185–91.
51. Gleicher N, Weghofer A, Kim A, et al. The impact in older women of ovarian FMR1 genotypes and subgenotypes on ovarian reserve. PLoS One. 2012;7(3):e33638.
52. Haadsma ML, Groen H, Fidler V, et al. The predictive value of ovarian reserve tests for miscarriage in a population of subfertile ovulatory women. Hum Reprod. 2009;24(3):546–52.
53. Kwee J, Schats R, McDonnell J, et al. The clomiphene citrate challenge test versus the exogenous follicle-stimulating hormone ovarian reserve test as a single test for identification of low responders and hyper-responders to in vitro fertilization. Fertil Steril. 2006;85(6):1714–22.
54. Kwee J, Schats R, McDonnell J, et al. Evaluation of anti-Mullerian hormone as a test for the prediction of ovarian reserve. Fertil Steril. 2008;90(3):737–43.
55. Nelson SM, Lawlor DA. Predicting live birth, preterm delivery, and low birth weight in infants born from in vitro fertilisation: a prospective study of 144,018 treatment cycles. PLoS Med. 2011;8:e1000386.
56. Oudendijk JF, Yarde F, Eijkemans MJ, et al. The poor responder in IVF: is the prognosis always poor?: a systematic review. Hum Reprod Update. 2012;18(1):1–11.
57. Al-Azemi M, Killick SR, Duffy S, et al. Multimarker assessment of ovarian reserve predicts oocyte yield after ovulation induction. Hum Reprod. 2011;26(2):414–22.
58. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WH. A validated model of serum anti-Mullerian hormone from conception to menopause. PLoS One. 2011;6(7):e22024.
59. Khader A, Lloyd SM, McConnachie A, et al. External validation of anti-Mullerian hormone based prediction of live birth in assisted conception. J Ovarian Res. 2013;6(1):3.
60. La Marca A, Nelson SM, Sighinolfi G, et al. Anti-Mullerian hormone-based prediction model for a live birth in assisted reproduction. Reprod Biomed Online. 2011;22(4):341–9.
61. Nelson SM, Yates RW, Fleming R. Serum anti-Mullerian hormone and FSH: prediction of live birth and extremes of response in stimulated cycles–implications for individualization of therapy. Hum Reprod. 2007;22(9):2414–21.
62. Broer SL, Dolleman M, Opmeer BC, et al. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. Hum Reprod Update. 2011;17(1):46–54.
63. Broer SL, Mol BW, Hendriks D, et al. The role of antimullerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. Fertil Steril. 2009;91(3):705–14.
64. Broer SL, van Disseldorp J, Broeze KA, et al. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach. Hum Reprod Update. 2013;19(1):26–36.
65. Nelson SM, Anderson RA, Broekmans FJ, Raine-Fenning N, Fleming R, La Marca A. Hum Reprod.

2012;27(3):631-6. doi: 10.1093/humrep/der446. Epub 2012 Jan 11.
66. van Disseldorp J, Lambalk CB, Kwee J, et al. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. Hum Reprod. 2010;25(1):221–7.
67. Majumder K, Gelbaya T, Laing I, Nardo LG. The use of anti-Müllerian hormone and antral follicle count to predict the potential of oocytes and embryos. Eur J Obstet Gynecol Reprod Biol. 2010;150:166–70.
68. Scott Jr RT, Elkind-Hirsch KE, Styne-Gross A, et al. The predictive value for in vitro fertility delivery rates is greatly impacted by the method used to select the threshold between normal and elevated basal follicle-stimulating hormone. Fertil Steril. 2008; 89(4):868–78.
69. Seifer DB, Lambert-Messerlian G, Hogan JW, Gardiner AC, Blazar AS, Berk CA. Day 3 serum inhibin-B is predictive of assisted reproductive technologies outcome. Fertil Steril. 1997;67(1):110–4.
70. Seifer DB, Scott Jr RT, Bergh PA, et al. Women with declining ovarian reserve may demonstrate a decrease in day 3 serum inhibin B before a rise in day 3 follicle-stimulating hormone. Fertil Steril. 1999;72(1):63–5.
71. Eldar-Geva T, Ben-Chetrit A, Spitz IM, Rabinowitz R, Markowitz E, Mimoni T, et al. Dynamic assays of inhibin B, anti-Mullerian hormone and estradiol following FSH stimulation and ovarian ultrasonography as predictors of IVF outcome. Hum Reprod. 2005;20:3178–83.
72. Fanchin R, de Ziegler D, Olivennes F, et al. Exogenous follicle stimulating hormone ovarian reserve test (EFORT): a simple and reliable screening test for detecting 'poor responders' in in-vitro fertilization. Hum Reprod. 1994;9(9):1607–11.
73. Elting MW, Kwee J, Schats R, et al. The rise of estradiol and inhibin B after acute stimulation with follicle-stimulating hormone predict the follicle cohort size in women with polycystic ovary syndrome, regularly menstruating women with polycystic ovaries, and regularly menstruating women with normal ovaries. J Clin Endocrinol Metab. 2001;86(4): 1589–95.
74. Jayaprakasan K, Chan Y, Islam R, et al. Prediction of in vitro fertilization outcome at different antral follicle count thresholds in a prospective cohort of 1,012 women. Fertil Steril. 2012;98(3):657–63.
75. Nardo LG, Gelbaya TA, Wilkinson H, et al. Circulating basal anti-Mullerian hormone levels as predictor of ovarian response in women undergoing ovarian stimulation for in vitro fertilization. Fertil Steril. 2009;92(5):1586–93.
76. Ebner T, Sommergruber M, Moser M, et al. Basal level of anti-Mullerian hormone is associated with oocyte quality in stimulated cycles. Hum Reprod. 2006;21(8):2022–6.
77. Sabatini L, Zosmer A, Hennessy EM, et al. Relevance of basal serum FSH to IVF outcome varies with patient age. Reprod Biomed Online. 2008;17(1):10–9.
78. Tremellen K, Kolo M. Serum anti-Mullerian hormone is a useful measure of quantitative ovarian reserve but does not predict the chances of live-birth pregnancy. Aust N Z J Obstet Gynaecol. 2010;50(6):568–72.
79. Gleicher N, Weghofer A, Barad DH. Anti-Mullerian hormone (AMH) defines, independent of age, low versus good live-birth chances in women with severely diminished ovarian reserve. Fertil Steril. 2010;94(7):2824–7.
80. Massie JA, Burney RO, Milki AA, et al. Basal follicle-stimulating hormone as a predictor of fetal aneuploidy. Fertil Steril. 2008;90(6):2351–5.
81. Sahu B, Oztutrk O, Serhal P, Jayaprakasan K. Do ovarian reserve tests predict miscarriage in women undergoing assisted reproduction treatment? Eur J Obstet Gynecol Reprod Biol. 2010;153(2):181–4.
82. Katz-Jaffe MG, Surrey ES, Minjarez DA, et al. Association of abnormal ovarian reserve parameters with a higher incidence of aneuploid blastocysts. Obstet Gynecol. 2013;121(1):71–7.
83. Pigny P, Jonard S, Robert Y, et al. Serum anti-Mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. J Clin Endocrinol Metab. 2006;91(3):941–5.
84. Dewailly D, Gronier H, Poncelet E, et al. Diagnosis of polycystic ovary syndrome (PCOS): revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. Hum Reprod. 2011;26(11):3123–9.
85. Freeman EW, Sammel MD, Lin H, et al. Contribution of the rate of change of antimullerian hormone in estimating time to menopause for late reproductive-age women. Fertil Steril. 2012;98(5):1254–9.
86. Sowers MR, Zheng H, McConnell D, et al. Follicle stimulating hormone and its rate of change in defining menopause transition stages. J Clin Endocrinol Metab. 2008;93(10):3958–64.
87. Tehrani FR, Solaymani-Dodaran M, Tohidi M, et al. Modeling age at menopause using serum concentration of anti-Mullerian hormone. J Clin Endocrinol Metab. 2013;98:729–35.
88. Schuh-Huerta SM, Johnson NA, Rosen MP, et al. Genetic variants and environmental factors associated with hormonal markers of ovarian reserve in Caucasian and African American women. Hum Reprod. 2012;27(2):594–608.
89. The Practice Committees of the American Society for Reproductive Medicine, The Society for Assisted Reproductive Technology. Mature oocyte cryopreservation: a guideline. Fertil Steril. 2013;99(1):37–43.
90. Hill KA, Nadler T, Mandel R, et al. Experience of young women diagnosed with breast cancer who undergo fertility preservation consultation. Clin Breast Cancer. 2012;12(2):127–32.
91. Fanchin R, Schonauer LM, Righini C, et al. Serum anti-Mullerian hormone dynamics during controlled ovarian hyperstimulation. Hum Reprod. 2003;18(2): 328–32.
92. Li HW, Wong CY, Yeung WS, et al. Serum anti-mullerian hormone level is not altered in women using

hormonal contraceptives. Contraception. 2011;83(6): 582–5.
93. Deb S, Campbell BK, Pincott-Allen C, Clewes JS, Cumberpatch G, Raine-Fenning NJ. Quantifying effect of combined oral contraceptive pill on functional ovarian reserve as measured by serum anti-Müllerian hormone and small antral follicle count using three-dimensional ultrasound. Ultrasound Obstet Gynecol. 2012;39(5):574–80.
94. Fabregues F, Castelo-Branco C, Carmona F, et al. The effect of different hormone therapies on anti-mullerian hormone serum levels in anovulatory women of reproductive age. Gynecol Endocrinol. 2011;27(4):216–24.
95. van den Berg MH, van Dulmen-den BE, Overbeek A, et al. Comparison of ovarian function markers in users of hormonal contraceptives during the hormone-free interval and subsequent natural early follicular phases. Hum Reprod. 2010;25(6):1520–7.
96. Kallio S, Puurunen J, Ruokonen A, Vaskivuo T, Piltonen T, Tapanainen JS. Fertil Steril. 2012. doi: pii: S0015-0282(12)02446-6.
97. Deb S, Campbell BK, Clewes JS, Pincott-Allen C, Raine-Fenning NJ. The intra-cycle variation in the number of antral follicles stratified by size and in the endocrine markers of ovarian reserve in women with normal ovulatory menstrual cycles. Ultrasound Obstet Gynecol. 2012;41:216–22.
98. Nelson SM, La Marca A. The journey from the old to the new AMH assay: how to avoid getting lost in the values. Reprod Biomed Online. 2011;23:411–20.
99. Rustamov O, Smith A, Roberts SA, et al. Anti-Mullerian hormone: poor assay reproducibility in a large cohort of subjects suggests sample instability. Hum Reprod. 2012;27(10):3085–91.
100. Streuli I, Fraisse T, Chapron C, et al. Clinical uses of anti-Mullerian hormone assays: pitfalls and promises. Fertil Steril. 2009;91(1):226–30.

Maternal Age and Oocyte Aneuploidy: Lessons Learned from Trisomy 21

6

Stephanie L. Sherman, Emily G. Allen, and Lora J.H. Bean

6.1 Introduction

In 1954, Penrose noted that the causes of Down syndrome (DS) must be heterogeneous due to the observed pattern of the maternal age association [1, 2]. Clearly, mothers of infants with DS were, on average, born to older mothers. However, about one-third of the individuals with DS in his series were not associated with maternal age [3]. He also observed that the mean maternal age was lower in families with two children with DS when compared with the general sample of children with DS. Based on his keen observations, he proposed several plausible causes of DS: genetic susceptibility, unbalanced chromosomes caused by translocation, and factors associated with fluctuating endocrine disturbance.

With the introduction of karyotyping, the etiology of DS was shown to be due to an extra chromosome 21, either standard trisomy 21 with three independent chromosomes 21 or a translocation chromosome with all or part of chromosome 21 translocated to another chromosome [4–7]. It is now estimated that approximately 95 % of individuals with DS have an extra chromosome 21 as a result of meiotic nondisjunction, or the abnormal segregation of chromosomes during gamete formation. Of the remaining 5 %, less than 1 % is due to somatic mosaicism (a portion of cells with the normal 46 chromosomes and the other line with 47 chromosomes) and the rest to chromosome 21 translocations [8].

Standard trisomy 21 has become an important model to understand meiotic nondisjunction in humans, as it is one of the few aneuploid conditions (having too many or too few chromosomes) that survives to term and can be relatively easily diagnosed at birth. The vast majority of fetuses with aneuploidy are lost during pregnancy. Even those with trisomy 21, the smallest human chromosomes containing approximately 1.5 % of the genome, are estimated to be lost about 50–80 % of the time [9, 10]. Infants born with trisomy 21have significant developmental and intellectual disabilities, birth defects, and later onset medical conditions associated with the extra dosage of chromosome 21 genes. Taken together, meiotic nondisjunction in humans is the leading cause of pregnancy loss and birth defects. It can be argued that meiotic nondisjunction is an important limiting factor in women's reproductive life span.

The association of advanced maternal age with trisomy has been noted for almost all human chromosomes. Clear evidence shows that this age effect is limited to nondisjunction errors that occur in the oocyte [11–14]. This conclusion is based on the following evidence. The age of the mother is not associated with: (1) a nondisjunction error in spermatogenesis (paternal errors [14–16]), (2) a post-zygotic mitotic error

S.L. Sherman, Ph.D. (✉) • E.G. Allen, Ph.D. • L.J.H. Bean, Ph.D.
Department of Human Genetics, Emory University School of Medicine, 615 Michael St, Atlanta, GA 30322, USA
e-mail: ssherma@emory.edu; emgrave@emory.edu; ljbean@emory.edu

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_6, © Springer Science+Business Media New York 2013

[14, 17], or (3) a translocation (inherited or de novo) [18]. Furthermore, in vitro fertilization procedures show that oocytes donated from a young woman to an older recipient result in embryo implantation and pregnancy rates expected for the donor's age. Thus, the aging oocyte, and not the aging uterine environment, is implicated as the risk factor for maternal nondisjunction [19].

There are critical differences between the process of oocyte and sperm development that influence susceptibility for meiotic nondisjunction. To start, meiosis including the two specialized cell divisions that lead to the haploid complement in gametes works on a very different timeline in oogenesis compared with spermatogenesis. Meiosis starts with an initial step of DNA replication and the formation of sister chromatid cohesion complex. In prophase I, homologous chromosomes synapse and recombination occurs. Recombination helps to tether chromosome pairs together along with sister chromatid cohesion. These pairs of homologous chromosomes then separate at the end of meiosis I (MI), whereas sister chromatids separate in meiosis II (MII). In men, spermatogenesis begins after puberty and cells entering meiosis move from one stage to the other without delay. In contrast, meiosis in women begins during fetal development and is arrested in prophase I after chromosomes synapse and recombine. MI resumes in the woman's adult life just before the ovulation of an oocyte. At this point, MI is completed and the first polar body is extruded. MII begins but arrests for a short period as the oocyte travels down the fallopian tubes. MII is completed after fertilization and the second polar body is extruded. Thus, meiosis in a woman extends over a 10- to 50-year period; the age of the woman at conception reflects that age of the oocyte, and primarily the period of arrest in MI.

Given the mechanistic differences and temporal separation of MI and MII, many have hypothesized that the risk factors for MI and MII nondisjunction errors are different. The ability to classify errors by parental and stage of origin of the meiotic event allowed the potential to test this hypothesis. This was first done using chromosome heteromorphic markers [20]. With the advent of DNA variant markers, this classification became more accurate and also allowed the potential to examine recombination profiles [21]. We took advantage of this technology and conducted two population-based case/controls studies whose aims were to understand the causes and consequences of trisomy 21: Atlanta Down Syndrome Project (ASDP) and National Down Syndrome Project (NDSP).

Data for ADSP was collected from 1989 to 1999 in cooperation with the Centers for Disease Control and Prevention (CDC) [16]. Cases were identified through a birth defect surveillance system and included liveborn infants with documented trisomy 21 or mosaic trisomy 21 born to women in the five-county Atlanta metropolitan area. Controls were randomly selected from newborns without birth defects in the same population. In 2000, we expanded the ADSP to five additional sites with established birth surveillance systems supported by CDC in the creation of the NDSP. For this population-based case/control series, birth years included 2000–2004 [22]. Through ADSP, we enrolled 308 infants with DS and their parents (77 % participation rate) along with 398 controls (60 % participation). Through NDSP, we recruited 907 infants identified with DS and their parents (61 % participation rate). For controls, 977 were enrolled (57 % participation rate). Additional convenient samples have also been collected to ask specific questions about recombination profiles.

Currently, studies that use genetic markers to separate maternal meiosis I (MI) and maternal meiosis II (MII) nondisjunction errors only vary in the genetic markers used; the basic assay is the same [22]. Although this classification is not perfect, it does begin to provide a way to examine less heterogeneous etiologic-based groups. A brief review of the method used in our studies will help show the limitation of the classification. First, the parental origin of the meiotic error is determined by establishing the contribution of parental alleles to the proband with trisomy 21. Once maternal origin of the meiotic error is established, markers located in the pericentromeric region (for our studies, ~13.6 to ~16.8 Mb) of 21q are used to infer the type of the meiotic error,

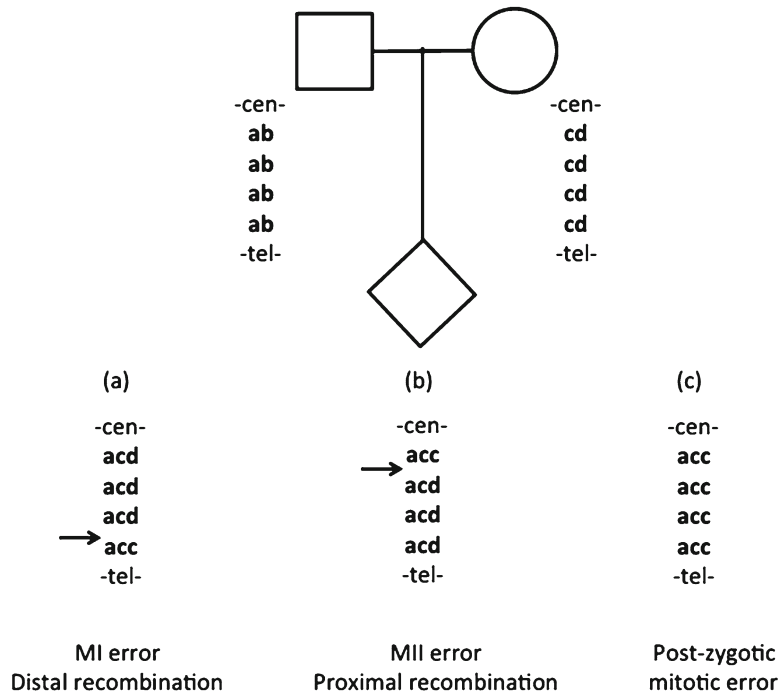

Fig. 6.1 Example of the method to determine type of nondisjunction errors and recombination profile. For the father, mother, and offspring with trisomy, a list of genotypes (alleles a, b, c, and d) from four small tandem repeat (STR) markers ordered from the centromere (-cen-) to the telomere (-tel-) are provided. Three examples are shown: (**a**) maternal meiosis I (MI) error with a recombinant between the third and fourth marker (*arrow*); (**b**) maternal meiosis II (MII) error with a recombinant between the first and second marker (*arrow*); and (**c**) a post-zygotic mitotic error

MI or MII. As shown in Fig. 6.1, if maternal heterozygosity is retained in the trisomic offspring, we assign an MI error. If maternal heterozygosity is reduced to homozygosity, we assign an MII error. When all informative markers along chromosome 21 are reduced to homozygosity, the origin of nondisjunction is most likely due to a post-zygotic, mitotic error. We have excluded these from our studies. This genetic assay cannot distinguish between the different types of underlying errors that might lead to an MI or MII error (Fig. 6.2). For example, sister chromatids that fail to separate during anaphase of MII or an error that is initiated in MI and not resolved properly in MII both lead to the contribution of sister chromatids to the oocyte. Also, if sister chromatids prematurely separate in MI and randomly segregate in MII, some configurations will lead to both sister chromatids segregating to the same pole in MII. Despite the limitations of assigning nondisjunction events as MI or MII errors, we and others have shown that this classification system can provide insight into the heterogeneous causes of nondisjunction during oocytes formation and allow us to test whether MI- or MII-specific modifiable factors could be targets of intervention.

6.2 Advanced Maternal Age is Associated with Both MI and MII Nondisjunction Errors

The link between DS and maternal age was first reported by Penrose et al. in 1933 [1]. Once it was possible to separate MI and MII using chromosome 21 specific genetic variants, it was clear that both types of errors were associated with maternal age [11, 12, 16, 22, 23]. Data from the combined ADSP and NDSP population-based

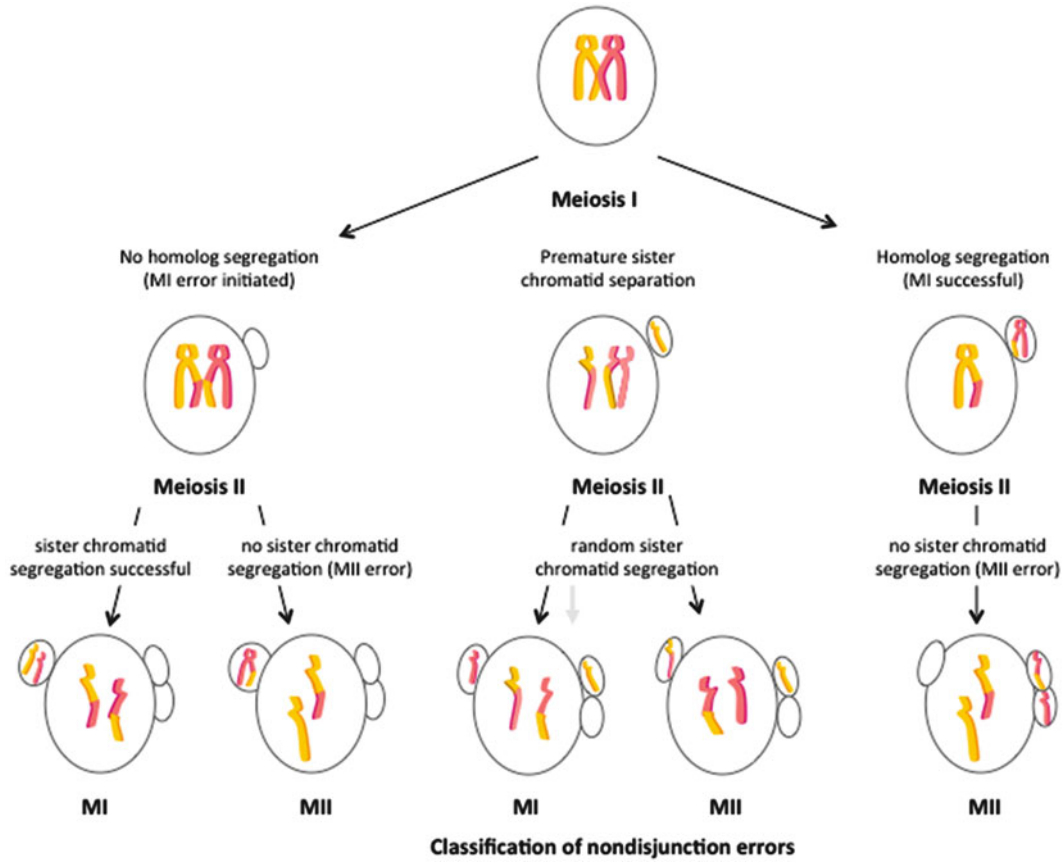

Fig. 6.2 Classification of meiosis I (MI) and meiosis II (MII) nondisjunction errors. Various types of nondisjunction errors are shown for a single pair of homologous chromosomes with a single exchange. Colors represent genetic markers that are used to classify the error and the recombination profile. The crook in the chromatid arm represents the centromeric/pericentromeric region which is used to classify the MI or MII error

studies of 1,215 mothers with an infant with DS compared to 1,375 control mothers illustrate this finding [23]. First, compared to mothers of controls, mothers with an MI error were 8.5 times more likely to be ≥40 years old than 20–24 years old at the birth of the index case (95 % CI = 5.6–12.9). Where nondisjunction was classified as an MII error, mothers were 15.1 times more likely to be ≥40 years (95 % CI = 8.4–27.3). As a consequence, the ratio of MI to MII errors differed by maternal age among women with a nondisjunction event: the ratio was lower among women <19 years of age and those ≥40 years (2.1, 2.3, respectively) and higher in the middle age group (3.6). Figure 6.3 shows the age distribution among mothers at the time of the birth of their infant with DS stratified by MI and MII errors to illustrate these findings. These data demonstrate that maternal age is the dominant risk factor for nondisjunction and all other investigations of nondisjunction risk factors must be carried out in the context of maternal age.

6.3 Nondisjunction-Associated Recombination Patterns Differ Significantly Among MI and MII Errors

Along with sister chromatid cohesion, the physical structure surrounding an exchange of chromosome material, a chiasma, provides the

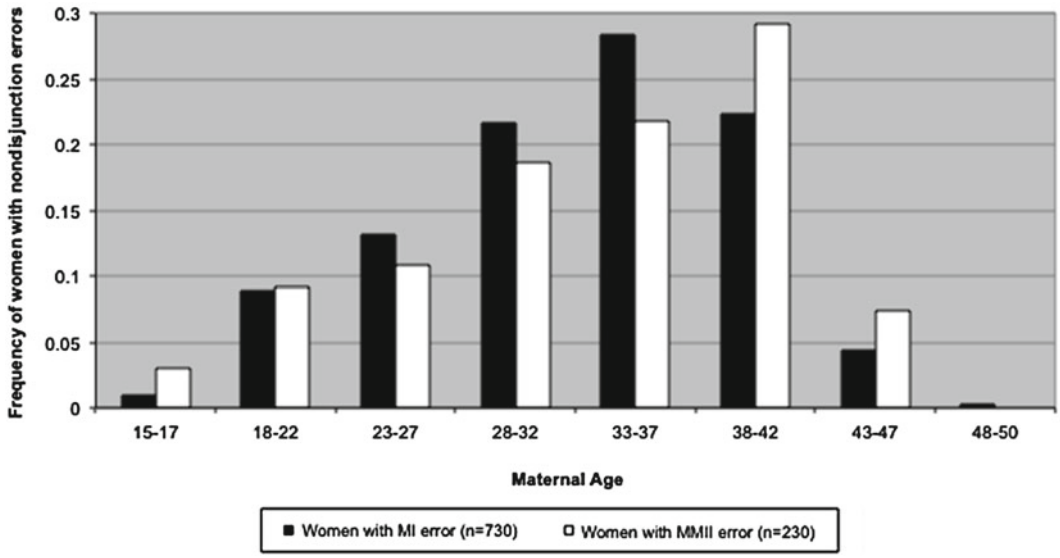

Fig. 6.3 Maternal age frequency distribution of women with maternal meiosis I (MI) or meiosis II (MII) nondisjunction errors

physical connection necessary for accurate chromosome segregation during MI [24]. Studies in model organisms have provided the basis to examine recombination as a risk factor for nondisjunction. These studies indicated that absent or reduced levels of recombination, along with suboptimally placed recombinant events (those too close to the centromere or too close to the telomere), increase the likelihood of nondisjunction [25–31]. Now, altered meiotic recombination patterns have been associated with nondisjunction of almost all human chromosomes studied to date [32]. Importantly, we found MI- and MII-specific recombination patterns associated with chromosome 21 nondisjunction. For maternal MI-derived trisomy 21, we estimated from recombination profiles along the nondisjoined chromosome that 40–47 % of MI cases are derived from oocytes with no meiotic exchange [33–35]. Furthermore, of those maternally derived MI cases with a single exchange, the majority of exchanges occur in the distal 6.5 Mb of chromosome 21. Intriguingly, MII errors are highly associated with pericentromeric exchanges [33, 34]. This apparent effect of an MI process—recombination—on MII nondisjunction suggests that at least a portion of so-called MII errors may have their origin in MI.

The link between recombination and human nondisjunction prompts the obvious question: what insight can be gained about the mechanism of nondisjunction by examining altered recombination patterns by maternal age? At first blush, we would hypothesize that altered recombination patterns would be independent of maternal age, as recombination occurs during the fetal stage of a woman. However, we and others have found that only some patterns of recombination along the nondisjoined chromosome are independent of the age of the oocyte, while others are not [35–38].

Prior to describing results, we will provide the framework for our approach and the interpretation of potential risk factors for nondisjunction (e.g., recombination pattern, genetic factors, environmental factors) in the context of maternal age. First, we and others have stratified by maternal age group using either two age groups (mothers <35 years at time of birth of their infant with trisomy 21 and those ≥35) or three age groups (<29, 29–34, >34 years). This is necessary as we hypothesize that nondisjunction mechanisms differ among younger and older women. When we consider a second risk factor, such as recombination pattern, in addition to maternal age, the interpretation is sometimes not intuitive as pointed out by Ghosh et al. [39]. When a second risk

factor is negatively associated with maternal age group, it suggests that second risk factor's effect on nondisjunction is independent of age. A factor that is positively correlated with maternal age group implies that there is a causal relationship between the factors or an interaction that exacerbates each effect. Both these interpretations assume that there is independence between maternal age and the risk factor in controls. Studies below will help to clarify this point.

We hypothesized that the lack of an exchange would increase the risk for nondisjunction, regardless of maternal age. However, results are more complex. Oliver et al. [35] found that the proportion of MI errors with no exchange was the highest among the youngest maternal age group (<29 years) compared with the other two age groups (29–34 and >34 years), indicating a maternal-age independent mechanism. However, the proportions did not decrease linearly with age as expected for an age-independent mechanism: the older age group had a nonsignificantly higher frequency compared with the middle age group. Ghosh et al. [38] found that the middle age group, not the youngest, had the highest proportion of nondisjoined chromosomes with no recombination. Perhaps these results provide preliminary evidence for a secondary backup mechanism that helps to distribute non-exchange bivalents and which is age dependent. There is evidence for such systems in Drosophila [40] and yeast [41]. Proteins in humans that appear to have a similar function as those in yeast that are involved in the proper segregation of non-exchange homologues have been shown to be downregulated with increasing ovarian age [42, 43]. Thus, the age-dependent down-regulation of essential proteins may lead to the decreased ability to properly segregate non-exchange chromosomes in aging oocytes. However, this is only speculation at this point and sampling variation is always a possibility. More data are needed to determine significance of these findings.

This complex pattern for non-exchange chromosomes is in contrast to those with a susceptible single distal exchange during MI. Studies show a clear decreasing frequency of distal recombinants along the nondisjoined chromosomes 21 with maternal age [34, 36, 38]. Thus, this chiasma configuration appears to confer the same risk for nondisjunction regardless of age. Susceptibility is most likely related to the minimal amount of the sister chromatid cohesion complex remaining distal to the exchange event [29, 44–48].

For MII errors, a maternal age-association pattern is apparent: susceptible pericentromeric exchanges, whether in the context of a single recombinant or multiple recombinants, occur at higher frequency in older oocytes compared with younger ones [34, 36, 38]. This observation could be explained in two ways: (1) a pericentromeric exchange initiates or exacerbates the susceptibility to maternal age risk factors or (2) a pericentromeric exchange protects the bivalent against age-related risk factors allowing proper segregation of homologues at MI, but not segregation of sisters at MII. We think that the former explanation is more consistent with the observed data. Our thought is based on the assumption that bivalents with multiple recombinants should be more resistant to age-related degradation; however, those with a pericentromeric exchange are not.

6.4 Genetic Risk Factors: Genome-Wide Recombination Difference Provides Preliminary Evidence for Genes Influencing Nondisjunction

Given the importance of chiasmata, the physical structure related to recombination and proper segregation, we would expect the number and location of exchanges to be tightly regulated in normal meiotic events in humans. However, studies using direct and indirect approaches reveal significant interindividual variation in genome-wide recombination rates, a phenomenon coined the "mother" effect [49–52]. In addition, data from Kong et al. [51] uncovered a "gamete" effect; in other words, they found that the number of recombinants was positively correlated among chromosomes within the same oocyte, even after adjusting for the "mother" effect. Although the basis for this variation is unclear, it points to a factor with global influence on recombination rates among most chromosomes simultaneously.

Findings from Brown et al. [53] provided preliminary data to support a gamete effect in oocytes with a nondisjoined chromosome 21. They found reduced recombination in the total genome of an oocyte with a MI-derived nondisjoined chromosome 21 and no detectable recombination. This reduction appeared to be consistent with the normal variation in recombination observed among oocytes. That is, there was a linear increase in the mean genome-wide recombination counts depending on the inferred number of exchanges along the chromosome 21. This observed pattern suggested that specific chromosomes may be at higher risk for nondisjunction when the number of genome-wide recombination events is less than some threshold.

The findings of Brown et al. [53] were based on limited number of oocytes with MI nondisjunction errors ($n = 15$). Thus, we have recently followed up these preliminary findings in a larger sample of 94 MI nondisjunction events (Middlebrooks et al., in preparation). We used the Golden Gate linkage panel on our extended DS proband families (trios, maternal grandparents, and siblings) to obtain genome-wide recombination profiles. We examined both the gamete effect, or the effect leading to a correlation of the number or placement of recombinants among chromosomes within a gamete, and the mother effect, or the effect that leads to a correlation of recombination patterns among oocytes from a single mother. Based on 94 proband families with an MI error and our preliminary analysis, we found evidence for overall reduced genome-wide recombination counts, irrespective of the number of chromosome 21 recombinants (i.e., lack of a gamete effect). Although data are limited, we find no evidence for a "mother effect" when comparing oocytes with a nondisjoined chromosome to those with normal disjunction. Although we are cautious with our interpretation of the data at this point, we speculate that there may be dysregulation of genome-wide recombination in the oocytes with a nondisjoined chromosome.

Could this dysregulation be due to genetic effects? Certainly, variations in genes involved in regulation of recombination patterns in normal meiotic events are prime candidates. Perhaps variants in genes that control recombination number (*RNF212* [54–56] and the inversion on 17q [55, 57]) and those involved in preference of recombination location (*PRDM9* [58–60]) would be the first to explore.

6.5 Environmental Risk Factors: Associations with Specific Types of Nondisjunction Errors

Many potential genetic and environmental risk factors have been and continue to be investigated for chromosome nondisjunction. Nagaoka et al. [61] provide an excellent review of potential biological mechanisms behind the increased risk of nondisjunction with increasing maternal age. One potential factor involved in the reduction of oocyte quality over time is the accumulation of toxic elements from the environment that could damage the meiotic machinery [62–64]. Oocytes are exposed to toxic influences that depend on the chronological age of the oocyte, lifestyle, and environment. Along with the intrinsic aging process of the oocyte, diminished oocyte quality could result from damage by reactive oxygen species (ROS) from metabolism [65]. Given the timeline of oogenesis, exposures impacting oocyte quality could occur during the fetal period of the woman (grandmaternal effect) or at any point in her lifetime before fertilization.

Environmental risk factors other than maternal age have been difficult to identify. Some of this is due to the inherent problem of defining exposures, pinpointing the timing of their effect, and capturing accurate information. In the case of nondisjunction, this may also be due, in part, to the heterogeneous types of errors that occur. Different components of the meiotic machinery or the specific processes involved in segregating homologues in MI and sister chromatids in MII may be more or less vulnerable to different insults or exposures. Recent studies have provided evidence to support this idea. We will review factors that have been explored as potential environmental risks for the different types of chromosome 21 nondisjunction errors, starting with the complex construct of social economic status (SES), and

then presenting data on specific exposures including use of tobacco products, oral contraceptives, and folic acid supplementation. Results are still preliminary, but begin to provide insight into the next steps that should be taken to hone in on causes of nondisjunction and identification of possible modifiable factors.

6.5.1 Social Economic Status

Torfs and Christianson [66] were the first to consider maternal socioeconomic status (SES) as a proxy for environmental exposures to predict the risk of a clinically recognized pregnancy with DS. The SES level of the mother was assessed both during her fetal development (e.g., the mother's father's occupation at the time of her birth) and during her lifetime before conception (e.g., the mother's level of education). They found an association between a clinically recognized pregnancy with DS and low maternal SES, after adjusting for race/ethnicity, gravidity, and maternal age. Specifically, the association with DS was significantly higher when the mother had less than a high school education, the father was employed as a laborer or unemployed, and the household income was less than $20,000. In a follow-up study, the impact of these SES risk factors differed by the type of maternal meiotic error: mothers who had a MII nondisjunction error were more likely to have a history of low SES [67].

To further study this effect, we used our data on 1,691 families ascertained through the NDSP [68]. We assessed MI and MII nondisjunction errors in the presence of three low SES factors: both parents had not completed high school, both maternal grandparents had not completed high school, and an annual household income of <$25,000. Using logistic regression models, adjusting for maternal age and race/ethnicity, an association was found with MII errors only, thus confirming the findings of Christianson and Torfs [67]. More specifically, MII chromosome 21 nondisjunction was more common among mothers with one low SES factor (OR = 1.81, 95 % CI = 1.07–3.05) and ≥2 low SES factors (OR = 2.17, 95 % CI = 1.02–4.63) compared to no low SES factors. This association was driven primarily by having a low household income (OR = 1.79, 95 % CI = 1.14–2.73). No difference was found in the ORs when mothers were stratified by maternal age (<35 or ≥35 years). Again, no association was detected among maternal MI errors. This significant association of low maternal SES, primarily driven by household income, being limited to MII cases suggests that SES factors associated with access to prenatal healthcare, prenatal diagnosis, and differential use of diagnostic information by the healthcare professionals or mothers cannot explain these results. If these were involved, the association would be significant among both types of maternal meiotic errors. However, the biological mechanism underlying the effect of low maternal SES exposure on MII nondisjunction is unclear. As SES is a proxy for environmental exposures, our results suggest that some environmental factors may influence only specific stages of meiosis.

6.5.2 Use of Tobacco Products and Oral Contraceptives Around the Time of Conception

Over the past years, researchers have been examining more specific environmental exposures to determine their influence on nondisjunction in the context of maternal age. A number of studies reported a nonsignificant negative association between maternal smoking around the time of conception and the risk for DS [69–74]. One possible explanation for the negative association was that smoking created an unfavorable intrauterine environment and trisomic conceptuses were selectively lost prenatally among women who smoke [70, 71]. However, other studies concluded that there is no association between DS and periconceptional smoking [75–77].

Using our ADSP cohort, Yang et al. [78] analyzed periconceptional smoking among women with MI and MII errors separately. They found that current smoking showed a nonsignificant negative association among mothers with MI errors (OR = 0.72; 95 % CI = 0.40–1.29) and

nonsignificant positive association among mothers with MII errors (OR = 1.55; 95 % CI = 0.64–3.76). Restricting the study to younger mothers (<35 years) led to stronger associations: MI OR = 0.69 (95 % CI = 0.35–1.37) and MII OR = 2.98 (95 % CI = 1.01–8.87). Our unpublished data from a later NDSP cohort showed a similar pattern, but ORs were also nonsignificant. Specifically for young mothers with MI and MII errors, the OR adjusted for maternal age, race/ethnicity, ascertainment site, and education was 0.746 (95 % CI = 0.425–1.311) and 1.361 (95 % CI = 0.537–3.454), respectively.

In Yang et al., we also examined use of oral contraceptives (OC) around the time of conception as another possible factor that compromises the environment of the follicle. We did not find an association in any of the meiotic groups [78]. However, a significant interaction of OC use and smoking was observed, but again limited to mothers with MII errors leading to an OR of 5.82 (95 % CI = 1.28–26.4). As with smoking, this effect was most marked among mothers <35 years (OR = 7.62; 95 % CI = 1.63–35.6). Data from mothers of MI errors did not show a significant association with OC use or with its interaction with smoking. Using our unpublished data from NDSP, the same pattern was observed: among young mothers with MII errors, the interaction between smoking and oral contraception use around the time of conception was the strongest, although not statistically significant (OR = 8.72; 95 % CI = 0.969–78.384).

Overall, data from both cohorts showed the same patterns of associations for smoking and oral contraceptive use around the time of conception; however, sample sizes of these unique set of women (i.e., those who smoked and used OCs around the time of conception) were small. Thus, it was important to confirm these results in an independent study. Recently, a study was conducted in the surrounding region of Kolkata, India. Ghosh et al. [39] used a similar design and similar environmental exposures: they studied smokeless chewing tobacco and oral contraceptive use around the time of conception. Oral contraceptive use was usually described as a short-term, irregular dose of pills on interview. They studied the interaction of these risk factors with maternal age and also examined recombination patterns. Here we will only present their findings comparing MI vs. MII cases, but we refer the reader to their paper for other interesting findings.

Briefly, they found a borderline significant association of smokeless tobacco use with MII (vs. MI) ($p = 0.08$), and this association was strongest in the young age group ($p = 0.006$ for age by smokeless chewing tobacco interaction). There was a gradual decrease in the proportion of smokeless chewing tobacco users among women with MII errors with increasing age (0.93, 0.64, and 0.5, respectively, for young, middle, and old users), while controls frequencies did not differ by age group (~40 %).

For the OC use, the pattern was different. There was a gradual increase in the proportion of oral contraceptive users with age of mothers with MI errors (0.15, 0.33, and 0.5 for younger (≤28 years), middle (29–34 years), and older (>35 years) users, respectively) and for MII errors (0.29, 0.35, and 0.67, respectively). Again, for controls the OC use was constant with age.

Lastly, Ghosh et al. [39] found an increase in the proportions of women who used both smokeless chewing tobacco and OCs with increasing age in each meiotic outcome group (with frequencies of risk-positive cases of 0.11, 0.18, and 0.33, respectively, for young, middle, and old users for MI and of 0.21, 0.29, and 0.5, respectively, for MII).

Taken together, the combined results from Yang et al. and Ghosh et al. suggest that chewing or smoking tobacco is an age-independent risk factor for MII errors. Although there was no evidence for an association of nondisjunction and OC use in Yang et al., there was a strong maternal age-dependent association in both MI and MII errors in the study of Ghosh et al. for OC use. They suggest that the intermittent use of the hormone medication may be more toxic than regular use. Nevertheless, when both risk factors were present, they showed a strong interaction effect although data are mixed with respect to the action being age independent or dependent. We think further study is warranted to resolve the conflicting

findings and to better understand the related mechanisms.

Ghosh et al. took the next important step and put their data in the context of recombination pattern. Briefly, use of smokeless chewing tobacco was associated with lack of recombination ($p=0.007$) among younger and middle-aged mothers with MI errors ($p=0.009$ for interaction). No association was observed with OC use and lack of recombination. For location of recombination, no associations were observed for either risk factor in either meiotic outcome group.

6.5.3 Folic Acid

Folic acid is a vital nutrient that supplies the single carbon molecules critical for basic cellular functions such as synthesis and methylation of both DNA and proteins [79]. Methylation of histones and pericentromeric DNA is critical for centromeric function [80]. Mutations in human genes critical for proper centromeric methylation such as the methylatransferase gene *DNMT3B* and the transcription factor *ZBTB24* are associated with centromere instability [81–84].

Numerous studies have associated maternal polymorphisms in the folate pathway genes with the risk of having a child with DS. In 1999 James et al. first observed an increase in the *MTHFR* c.677C>T allele in mothers of children with DS in a US population [85]. Studies conducted in a wide range of North American, South American, European, and Asian populations found significant associations with either *MTHFR* c.677C>T, c.1298A>C, *MTR* c.2756C>G, *MTRR* c.66A>G, *CBS* 844ins68, and/or *SLC19A1* (also known as *RFC-1*) c.80A>G and the risk of having a child with DS. These studies were extensively reviewed in [86] and can be combined with more recent studies [87–91]. For example, a recent study on another folate pathway-related gene, the DNA methyltransferase gene *DNMT3B,* found an association between maternal promoter polymorphisms and DS [87]. However, some studies have reported no significant association between maternal folate pathway polymorphisms and the risk of having a child with DS [92–96]. In many studies with positive findings, statistical significance was either attained or enhanced by analysis of combinations of poylmorphisms. These data suggest that multiple hits to the folate pathway increase the risk of nondisjunction, including gene–environment interactions. For example, Chango et al. suggest that the folate-rich French diet may ameliorate the effects of folate gene polymorphisms. Clearly the mechanisms governing efficiency of folate metabolism are complex and depend upon both genetic and environmental factors.

In 1992 the US Public Health Service (USPHS) issued a recommendation that all women who may become pregnant take a folic acid supplement [97]. The USPHS went on to mandate fortification of enriched cereal-grain products, which was optional in 1996 and 1997 and required in 1998 [98, 99]. If maternal folic acid supplementation lowers the risk for chromosome 21 nondisjunction, one would expect the prevalence of DS in the USA to drop after the 1998 mandate for folic acid fortification; however, this has not happened to date [100–103]. There are several competing factors that make the design and interpretation of these studies difficult.

The use of noninvasive prenatal screening for trisomy and other aneuploidies increased through the 1990s. Collins et al. report no change in the proportion of trisomy 21 cases and a slight increase in other aneuploidies in cases referred for cytogenetic testing in South Carolina from 1990 to 1999. However, other factors are changing that can mask any potential effect. For example, maternal age at delivery is steadily increasing [104, 105]. With the implementation of maternal serum screening, a noninvasive test offered to all pregnant women, more women are being tested for a fetus with DS. The former predicts an increase in conceptions with DS and the latter a decrease in live births with DS.

Another way to examine the effect of the folate pathway is to study the use of folic acid supplementation during pregnancy. Only a few studies have done so. It is important to note that although some of the population-based studies corrected for maternal age [100, 103, 106] none of these studies stratified their population by

maternal age and, therefore, did not examine the effect of folic acid supplementation on older versus younger mothers. In addition, the longest any of these studies extend is to births in 1999 or 2000, capturing at most 2 or 3 years post-fortification. The length of exposure to folic acid that would be needed to see a biological effort on chromosome segregation is, at this time, unknown. Of particular note, none of these studies differentiate trisomy 21 resulting from maternal MI, maternal MII, or paternal errors.

Using data from the NDSP, we compared the use of folic acid-containing supplements among mothers of infants with full trisomy 21 due to maternal nondisjunction ($n=702$) and mothers of infants born with no major birth defects ($n=983$) [107]. Adjusting for maternal age, race/ethnicity, and infant age at maternal interview, we found no evidence of an association between lack of folic acid supplementation and maternal nondisjunction among all case mothers (OR = 1.16; 95 % CI: 0.90–1.48). In analyses stratified by meiotic stage and maternal age (<35 years or ≥35 years), we found an association among older mothers experiencing MII nondisjunction errors (OR = 2.00; 95 % CI: 1.08–3.71). These data suggest that lack of folic acid supplementation may be associated specifically with MII errors in the aging oocyte. If confirmed, these results could account for inconsistencies among previous studies, as each study sample may vary by maternal age structure and proportion of meiotic errors.

As an exploratory analysis, we also examined the effect of limiting the MII case sample to those with a single pericentromeric recombination event, or those events associated with MII older oocytes (described above). Perhaps such pericentromeric recombination events are dependent upon centromeric proteins that degrade with age and are sensitive to environmental exposures. To test this, we examined MII cases with a single pericentromeric recombination event, irrespective of the age of the mother ($n=90$). The association between lack of maternal folic acid supplementation and a MII nondisjunction error in the presence of a single pericentromeric recombinant event was marginally significant (OR = 1.77; 95 % CI: 1.02–3.06; $p=0.02$). We further grouped cases by age of the mother. The OR was higher among older mothers (OR = 2.06; 95 % CI: 1.00–4.23; $p=0.02$; $n=63$) compared to younger mothers (OR = 1.14; 95 % CI: 0.46–2.82; $p=0.39$; $n=27$). These preliminary data suggest that the risk for improper segregation of bivalents with at risk recombination patterns could be exacerbated by environmental exposures such as lack of folic acid supplementation.

6.6 Telomere Length as a Surrogate Marker for the Aging Ovary

Replicative shortening of telomeres is a marker of biological aging. It may be true that it also serves as a marker for the biological age of ovary. Many hypotheses for the maternal age effect on nondisjunction are focused on the degraded properties of the aged ovary [108]. If true, we may expect to observe shorter telomeres among mothers with nondisjunction events compared to mothers without observed nondisjunction. Based on this prediction, Ghosh et al. [109] compared the telomere lengths of mothers of trisomic offspring (case mothers) to mothers with normal offspring (control mothers). They measured telomere length in peripheral blood lymphocytes among age-matched mothers of children with DS (cases: $n=75$) and mothers of children without DS (controls: $n=75$) in an age range of 18–42 years. They stratified cases by MI ($n=48$) and MII errors ($n=27$) and performed linear regression to compare telomere length as a function of age in the three meiotic outcome groups. They showed that all three groups had similar telomere lengths on average for younger mothers and all three groups declined with age. Interestingly, telomere loss was greatest in the MII case group and smallest in the control group, with the MI case group in between. These results do not support the hypothesis that young mothers with nondisjunction events have biologically older ovaries than their chronological age. However, the increased telomeres loss in later years may be a surrogate for (or parallel) an increase in age-related exposures that affect the ovarian

environment. The increased telomere loss in MII vs. MI errors is consistent with the findings above where maternal-age dependent exposures appear to play a larger role in MII nondisjunction.

6.7 Conclusions

It is of vital importance to understand the context of the studies reviewed here. First, all studies reviewed here are based on live born infants with standard trisomy 21, those conceptuses that survive to term. As noted above, this only provides a picture of the "tip of the iceberg." Second, only one oocyte from the case and control women is examined. Events related an index case "oocyte" that resulted in a live born infant with standard trisomy 21 are compared to an index control "oocyte" that resulted in a live born infant without trisomy or major birth defect. We speculate that the vast majority of women including "controls" have had at least one oocyte that harbored an extra chromosome 21 because of a nondisjunction event. Considering these caveats, it is impressive that risk factors still come to the forefront.

Maternal age remains the most significant risk factor associated with human chromosome nondisjunction—this effect is not restricted to chromosome 21 [110]. Clearly progress is being made to understand its targets using advanced technology and direct study of gametes [61]. In the studies reviewed here, we interpret risk factors as being maternal age independent or dependent. We have to appreciate that the oocytes we examine have already aged over some 15 years prior to entering the study. Nonetheless, patterns emerge when we separate nondisjunction errors into more homogeneous groups (Figs. 6.1 and 6.2). In addition to maternal age, it appears that environmental factors, including SES, use of tobacco products and oral contraceptives, and use of folic acid supplements, are more strongly associated with MII errors than MI errors as summarized in Table 6.1. Among cases of trisomy 21, MII errors are less common than MI errors; therefore, results

Table 6.1 Summary of possible environmental risk factors for MI and MII nondisjunction of chromosome 21 errors in the context of maternal age

Exposure	MI error	MII error
Low social economic status	No association	• Positive association • No difference when stratified by maternal age
Tobacco use:		
Maternal smoking	• Nonsignificant negative association • Increased in mothers <35 years; maternal age independent	• Nonsignificant positive association • Increased in mothers <35 years; maternal age independent
Smokeless tobacco use	• Positive association • Increased in mothers <28 years ; maternal age independent	• Positive association • Increased in mothers <28 years ; maternal age independent
Oral contraceptive use: Around conception	No association	No association
Intermittent OC use	• Positive association • Increased in older mothers; maternal age dependent	• Positive association • Increased in older mothers; maternal age dependent
Interaction between tobacco use and OC use: Smoking and OC use	No interaction	• Positive interaction • Increased in mothers <35 years; maternal age independent
Smokeless tobacco and intermittent OC use	• Positive interaction • Increased in older mothers; maternal age dependent	• Positive interaction • Increased in older mothers; maternal age dependent
Lack of folic acid supplementation	No association	• Positive association • Increased in mothers >35 years old; maternal age dependent

are often based on small numbers. Some associations are replicated in two independent populations suggesting that they are robust. We must still remain cautious, as the biological mechanism for the associations is not known (e.g., whether they are independent of the age of the oocyte or exacerbated by age).

It is striking that environmental risk factors are disproportionately associated with MII errors. This could be an artifact that results from our ability to collect accurate data on environment risk periods that affect MII versus MI errors. That is, exposures occurring around the time of ovulation and fertilization are more accurately defined and reported compared with exposures that may be important during the initiation of MI, which occur in the fetal life of the woman. The latter time period would depend on the environment of the grandmother of the infant with trisomy 21. However, it is important to remember that resumption of MI occurs around ovulation, and this process may also be vulnerable to the mother's environmental exposures

A next important step is to ask about environmental risk factors and their possible interaction with chiasma configurations. This important work has been initiated by Ghosh et al. [39] and Hollis et al. [107]. These studies will be complicated by classification errors related to using observed recombination profiles. For example, the group defined as having no observed recombinants includes bivalents with no exchanges (about 40 % of the sample) and those with undetectable exchanges. This particular group may be the one with the greatest misclassification, or "noise," compared with those classified by the closest proximal event. Irrespective, this group of MI errors with no recombination is extremely valuable to study and will take large samples to overcome the noise. The biological mechanism to segregate non-exchange chromosomes in humans has not been studied. Risk factors may play a different role if there is a unique mechanism for distributing these non-exchange bivalents, as there is in other organisms [40, 41]. Ghosh et al. found that younger oocytes with MI errors and no detectable recombinants were associated with use of tobacco products, indicting a maternal age-independent factor. This needs to be confirmed, but points to an important direction of study to examine risk factors by chiasma configuration.

As noted in the beginning of this discussion, the studies reviewed are those based on conceptuses that survived to term. We need to keep this in mind when interpreting associations as risk or as protective factors. Ideally, a study of women with conceptuses with trisomy 21 identified early in pregnancy should be conducted. This type of study could be performed given the noninvasive prenatal screening and diagnostic tools that are now available. However, such studies would incur significant emotional burden on women and would need to be considered carefully by the community prior to approval. Irrespective, important progress is being made using the current strategy.

References

1. Penrose LS. The relative effects of paternal and maternal age in mongolism. J Genet. 1933;27(1):219–24.
2. Penrose LS. The relative aetological importance of birth order and maternal age in Mongolism. Proc R Soc B Biol Sci. 1934;115:431–50.
3. Penrose LS. Observations on the aetiology of mongolism. Lancet. 1954;267(6837):505–9.
4. Book JA, Fraccaro M, Lindsten J. Cytogenetical observations in mongolism. Acta Paediatr. 1959;48:453–68.
5. Ford CE, Jones KW, Miller OJ, et al. The chromosomes in a patient showing both mongolism and the Klinefelter syndrome. Lancet. 1959;1(7075):709–10.
6. Jacobs PA, Baikie AG, Court Brown WM, et al. The somatic chromosomes in mongolism. Lancet. 1959;1(7075):710.
7. Le Mongolism LJ. Premier exemple d'aberration autosomique humaine. Ann Genet. 1959;1:41–9.
8. Mutton D, Alberman E, Hook EB. Cytogenetic and epidemiological findings in Down syndrome, England and Wales 1989 to 1993. National Down Syndrome Cytogenetic Register and the Association of Clinical Cytogeneticists. J Med Genet. 1996;33(5):387–94.
9. Hook EB, Mutton DE, Ide R, et al. The natural history of Down syndrome conceptuses diagnosed

prenatally that are not electively terminated. Am J Hum Genet. 1995;57(4):875–81.
10. Hassold TJ, Jacobs PA. Trisomy in man. Annu Rev Genet. 1984;18:69–97.
11. Muller F, Rebiffe M, Taillandier A, et al. Parental origin of the extra chromosome in prenatally diagnosed fetal trisomy 21. Hum Genet. 2000;106(3): 340–4.
12. Antonarakis SE, Petersen MB, McInnis MG, et al. The meiotic stage of nondisjunction in trisomy 21: determination by using DNA polymorphisms. Am J Hum Genet. 1992;50(3):544–50.
13. Ballesta F, Queralt R, Gomez D, et al. Parental origin and meiotic stage of non-disjunction in 139 cases of trisomy 21. Ann Genet. 1999;42(1):11–5.
14. Sherman SL, Freeman SB, Allen EG, et al. Risk factors for nondisjunction of trisomy 21. Cytogenet Genome Res. 2005;111(3–4):273–80.
15. Petersen MB, Antonarakis SE, Hassold TJ, et al. Paternal nondisjunction in trisomy 21: excess of male patients. Hum Mol Genet. 1993;2(10):1691–5.
16. Yoon PW, Freeman SB, Sherman SL, et al. Advanced maternal age and the risk of Down syndrome characterized by the meiotic stage of chromosomal error: a population-based study. Am J Hum Genet. 1996; 58(3):628–33.
17. Antonarakis SE, Avramopoulos D, Blouin JL, et al. Mitotic errors in somatic cells cause trisomy 21 in about 4.5 % of cases and are not associated with advanced maternal age. Nat Genet. 1993;3(2):146–50.
18. Hook EB. Down syndrome rates and relaxed selection at older maternal ages. Am J Hum Genet. 1983;35(6):1307–13.
19. Sauer MV. The impact of age on reproductive potential: lessons learned from oocyte donation. Maturitas. 1998;30(2):221–5.
20. Mikkelsen M, Hallberg A, Poulsen H. Maternal and paternal origin of extra chromosome in trisomy 21. Hum Genet. 1976;32:17–21.
21. Warren AC, Chakravarti A, Wong C, et al. Evidence for reduced recombination on the nondisjoined chromosome 21 in Down syndrome. Science. 1987; 237:652–4.
22. Freeman SB, Allen EG, Oxford-Wright CL, et al. The National Down Syndrome Project: design and implementation. Public Health Rep. 2007;122(1): 62–72.
23. Allen EG, Freeman SB, Druschel C, et al. Maternal age and risk for trisomy 21 assessed by the origin of chromosome nondisjunction: a report from the Atlanta and National Down Syndrome Projects. Hum Genet. 2009;125(1):41–52.
24. Page SL, Hawley RS. Chromosome choreography: the meiotic ballet. Science. 2003;301(5634):785–9.
25. Koehler KE, Boulton CL, Collins HE, et al. Spontaneous X chromosome MI and MII nondisjunction events in *Drosophila melanogaster* oocytes have different recombinational histories. Nat Genet. 1996;14(4):406–14.
26. Krawchuk MD, Wahls WP. Centromere mapping functions for aneuploid meiotic products: analysis of rec8, rec10 and rec11 mutants of the fission yeast *Schizosaccharomyces pombe*. Genetics. 1999;153(1):49–55.
27. Moore DP, Miyazaki WY, Tomkiel JE, et al. Double or nothing: a Drosophila mutation affecting meiotic chromosome segregation in both females and males. Genetics. 1994;136(3):953–64.
28. Rasooly RS, New CM, Zhang P, et al. The lethal(1) TW-6cs mutation of Drosophila melanogaster is a dominant antimorphic allele of nod and is associated with a single base change in the putative ATP-binding domain. Genetics. 1991;129(2):409–22.
29. Ross LO, Maxfield R, Dawson D. Exchanges are not equally able to enhance meiotic chromosome segregation in yeast. Proc Natl Acad Sci USA. 1996;93(10):4979–83.
30. Sears DD, Hegemann JH, Shero JH, et al. Cis-acting determinants affecting centromere function, sister-chromatid cohesion and reciprocal recombination during meiosis in *Saccharomyces cerevisiae*. Genetics. 1995;139(3):1159–73.
31. Zetka MC, Rose AM. Mutant rec-1 eliminates the meiotic pattern of crossing over in *Caenorhabditis elegans*. Genetics. 1995;141(4):1339–49.
32. Lamb NE, Sherman SL, Hassold TJ. Effect of meiotic recombination on the production of aneuploid gametes in humans. Cytogenet Genome Res. 2005;111(3–4):250–5.
33. Lamb NE, Feingold E, Savage A, et al. Characterization of susceptible chiasma configurations that increase the risk for maternal nondisjunction of chromosome 21. Hum Mol Genet. 1997;6(9):1391–9.
34. Lamb NE, Freeman SB, Savage-Austin A, et al. Susceptible chiasmate configurations of chromosome 21 predispose to non-disjunction in both maternal meiosis I and meiosis II. Nat Genet. 1996;14(4):400–5.
35. Oliver TR, Feingold E, Yu K, et al. New insights into human nondisjunction of chromosome 21 in oocytes. PLoS Genet. 2008;4(3):e1000033.
36. Oliver TR, Tinker SW, Allen EG, et al. Altered patterns of multiple recombinant events are associated with nondisjunction of chromosome 21. Hum Genet. 2012;131(7):1039–46.
37. Lamb NE, Yu K, Shaffer J, et al. Association between maternal age and meiotic recombination for trisomy 21. Am J Hum Genet. 2005;76(1):91–9.
38. Ghosh S, Feingold E, Dey SK. Etiology of Down syndrome: evidence for consistent association among altered meiotic recombination, nondisjunction, and maternal age across populations. Am J Med Genet A. 2009;149A(7):1415–20.
39. Ghosh S, Hong CS, Feingold E, et al. Epidemiology of Down syndrome: new insight into the multidimensional interactions among genetic and environmental risk factors in the oocyte. Am J Epidemiol. 2011;174(9):1009–16.

40. Knowles BA, Hawley RS. Genetic analysis of microtubule motor proteins in Drosophila: a mutation at the ncd locus is a dominant enhancer of nod. Proc Natl Acad Sci USA. 1991;88(16):7165–9.
41. Cheslock PS, Kemp BJ, Boumil RM, et al. The roles of MAD1, MAD2 and MAD3 in meiotic progression and the segregation of nonexchange chromosomes. Nat Genet. 2005;37(7):756–60.
42. Baker DJ, Jeganathan KB, Cameron JD, et al. BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. Nat Genet. 2004;36(7):744–9.
43. Steuerwald N, Cohen J, Herrera RJ, et al. Association between spindle assembly checkpoint expression and maternal age in human oocytes. Mol Hum Reprod. 2001;7(1):49–55.
44. Orr-Weaver T. Meiotic nondisjunction does the two-step. Nat Genet. 1996;14(4):374–6.
45. Angell R. Mechanism of chromosome nondisjunction in human oocytes. Prog Clin Biol Res. 1995; 393:13–26.
46. Angell RR, Xian J, Keith J, et al. First meiotic division abnormalities in human oocytes: mechanism of trisomy formation. Cytogenet Cell Genet. 1994; 65(3):194–202.
47. Hawley RS, Frazier JA, Rasooly R. Separation anxiety: the etiology of nondisjunction in flies and people. Hum Mol Genet. 1994;3(9):1521–8.
48. Koehler KE, Hawley RS, Sherman S, et al. Recombination and nondisjunction in humans and flies. Hum Mol Genet. 1996;5 Spec No: 1495–504.
49. Broman KW, Murray JC, Sheffield VC, et al. Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am J Hum Genet. 1998;63(3):861–9.
50. Cheung VG, Burdick JT, Hirschmann D, et al. Polymorphic variation in human meiotic recombination. Am J Hum Genet. 2007;80(3):526–30.
51. Kong A, Gudbjartsson DF, Sainz J, et al. A high-resolution recombination map of the human genome. Nat Genet. 2002;31(3):241–7.
52. Lynn A, Koehler KE, Judis L, et al. Covariation of synaptonemal complex length and mammalian meiotic exchange rates. Science. 2002;296(5576): 2222–5.
53. Brown AS, Feingold E, Broman KW, et al. Genome-wide variation in recombination in female meiosis: a risk factor for non-disjunction of chromosome 21. Hum Mol Genet. 2000;9(4):515–23.
54. Kong A, Thorleifsson G, Stefansson H, et al. Sequence variants in the RNF212 gene associate with genome-wide recombination rate. Science. 2008;319(5868):1398–401.
55. Chowdhury R, Bois PR, Feingold E, et al. Genetic analysis of variation in human meiotic recombination. PLoS Genet. 2009;5(9):e1000648.
56. Fledel-Alon A, Leffler EM, Guan Y, et al. Variation in human recombination rates and its genetic determinants. PLoS One. 2011;6(6):e20321.
57. Stefansson H, Helgason A, Thorleifsson G, et al. A common inversion under selection in Europeans. Nat Genet. 2005;37(2):129–37.
58. Kong A, Thorleifsson G, Gudbjartsson DF, et al. Fine-scale recombination rate differences between sexes, populations and individuals. Nature. 2010;467(7319):1099–103.
59. Berg IL, Neumann R, Lam KW, et al. PRDM9 variation strongly influences recombination hot-spot activity and meiotic instability in humans. Nat Genet. 2010;42(10):859–63.
60. Baudat F, Buard J, Grey C, et al. PRDM9 is a major determinant of meiotic recombination hotspots in humans and mice. Science. 2010;327(5967): 836–40.
61. Nagaoka SI, Hassold TJ, Hunt PA. Human aneuploidy: mechanisms and new insights into an age-old problem. Nat Rev Genet. 2012;13(7):493–504.
62. Pacchierotti F, Eichenlaub-Ritter U. Environmental hazard in the aetiology of somatic and germ cell aneuploidy. Cytogenet Genome Res. 2011;133(2–4): 254–68.
63. Penrose LS. Genetical aspects of mental deficiency. In: Proceedings of the international Copenhagen Congress on the scientific study of mental retardation; 1964. p. 165–72.
64. Susiarjo M, Hassold TJ, Freeman E, et al. Bisphenol A exposure in utero disrupts early oogenesis in the mouse. PLoS Genet. 2007;3(1):e5.
65. Eichenlaub-Ritter U, Wieczorek M, Luke S, et al. Age related changes in mitochondrial function and new approaches to study redox regulation in mammalian oocytes in response to age or maturation conditions. Mitochondrion. 2011;11(5):783–96.
66. Torfs CP, Christianson RE. Socioeconomic effects on the risk of having a recognized pregnancy with Down syndrome. Birth Defects Res A Clin Mol Teratol. 2003;67(7):522–8.
67. Christianson RE, Sherman SL, Torfs CP. Maternal meiosis II nondisjunction in trisomy 21 is associated with maternal low socioeconomic status. Genet Med. 2004;6(6):487–94.
68. Hunter JE, Allen EG, Shin M, Bean LJH, Correa A, Druschel C, Hobbs CA, O'Leary LA, Romitti PA, Royle MH, Torfs CP, Freeman SB, Sherman SL. The association of low socioeconomic status and the risk of having a child with Down syndrome: a report from the National Down Syndrome Project. Genet Med. 2013. Manuscript under submission.
69. Kline J, Levin B, Shrout P, et al. Maternal smoking and trisomy among spontaneously aborted conceptions. Am J Hum Genet. 1983;35(3):421–31.
70. Kline J, Levin B, Stein Z, et al. Cigarette smoking and trisomy 21 at amniocentesis. Genet Epidemiol. 1993;10(1):35–42.
71. Hook EB, Cross PK. Cigarette smoking and Down syndrome. Am J Hum Genet. 1985;37(6):1216–24.
72. Hook EB, Cross PK. Maternal cigarette smoking, Down syndrome in live births, and infant race. Am J Hum Genet. 1988;42(3):482–9.

73. Shiono PH, Klebanoff MA, Berendes HW. Congenital malformations and maternal smoking during pregnancy. Teratology. 1986;34(1):65–71.
74. Chen CL, Gilbert TJ, Daling JR. Maternal smoking and Down syndrome: the confounding effect of maternal age. Am J Epidemiol. 1999;149(5):442–6.
75. Torfs CP, Christianson RE. Effect of maternal smoking and coffee consumption on the risk of having a recognized Down syndrome pregnancy. Am J Epidemiol. 2000;152(12):1185–91.
76. Cuckle HS, Alberman E, Wald NJ, et al. Maternal smoking habits and Down's syndrome. Prenat Diagn. 1990;10(9):561–7.
77. Kallen K. Down's syndrome and maternal smoking in early pregnancy. Genet Epidemiol. 1997;14(1): 77–84.
78. Yang Q, Sherman SL, Hassold TJ, et al. Risk factors for trisomy 21: maternal cigarette smoking and oral contraceptive use in a population-based case–control study. Genet Med. 1999;1(3):80–8.
79. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. Cell. 2007;128(4):669–81.
80. Gonzalez-Barrios R, Soto-Reyes E, Herrera LA. Assembling pieces of the centromere epigenetics puzzle. Epigenetics. 2012;7(1):3–13.
81. Xu GL, Bestor TH, Bourc'his D, et al. Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. Nature. 1999;402(6758):187–91.
82. Hansen RS, Wijmenga C, Luo P, et al. The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. Proc Natl Acad Sci USA. 1999;96(25):14412–7.
83. Chouery E, Abou-Ghoch J, Corbani S, et al. A novel deletion in ZBTB24 in a Lebanese family with immunodeficiency, centromeric instability, and facial anomalies syndrome type 2. Clin Genet. 2012; 82(5):489–93.
84. de Greef JC, Wang J, Balog J, et al. Mutations in ZBTB24 are associated with immunodeficiency, centromeric instability, and facial anomalies syndrome type 2. Am J Hum Genet. 2011;88(6):796–804.
85. James SJ, Pogribna M, Pogribny IP, et al. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr. 1999;70(4):495–501.
86. Coppede F. The complex relationship between folate/homocysteine metabolism and risk of Down syndrome. Mutat Res. 2009;682(1):54–70.
87. Coppede F, Bosco P, Tannorella P, Romano C, Antonucci I, Stuppia L, et al. DNMT3B promoter polymorphisms and maternal risk of birth of a child with Down syndrome. Hum Reprod. (Oxford, England). 2013;28(2):545–50.
88. Brandalize AP, Bandinelli E, Dos Santos PA, et al. Maternal gene polymorphisms involved in folate metabolism as risk factors for Down syndrome offspring in Southern Brazil. Dis Markers. 2010;29(2): 95–101.
89. Fintelman-Rodrigues N, Correa JC, Santos JM, et al. Investigation of CBS, MTR, RFC-1 and TC polymorphisms as maternal risk factors for Down syndrome. Dis Markers. 2009;26(4):155–61.
90. Biselli JM, Zampieri BL, Goloni-Bertollo EM, et al. Genetic polymorphisms modulate the folate metabolism of Brazilian individuals with Down syndrome. Mol Biol Rep. 2012;39(10):9277–84.
91. Wang SS, Feng L, Qiao FY, Lv JJ. Functional variant in methionine synthase reductase decreases the risk of Down syndrome in China. The journal of obstetrics and gynaecology research. 2013;39(2):511–5.
92. Boduroglu K, Alanay Y, Koldan B, et al. Methylenetetrahydrofolate reductase enzyme polymorphisms as maternal risk for Down syndrome among Turkish women. Am J Med Genet A. 2004;127(1):5–10.
93. Chango A, Fillon-Emery N, Mircher C, et al. No association between common polymorphisms in genes of folate and homocysteine metabolism and the risk of Down's syndrome among French mothers. Br J Nutr. 2005;94(2):166–9.
94. Kokotas H, Grigoriadou M, Mikkelsen M, et al. Investigating the impact of the Down syndrome related common MTHFR 677C>T polymorphism in the Danish population. Dis Markers. 2009;27(6):279–85.
95. Mendes CC, Biselli JM, Zampieri BL, et al. 19-base pair deletion polymorphism of the dihydrofolate reductase (DHFR) gene: maternal risk of Down syndrome and folate metabolism. Sao Paulo Med J. 2010;128(4):215–8.
96. Neagos D, Cretu R, Tutulan-Cunita A, et al. Methylenetetrahydrofolate dehydrogenase (MTHFD) enzyme polymorphism as a maternal risk factor for trisomy 21: a clinical study. J Med Life. 2010;3(4):454–7.
97. CDC. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR Recomm Rep. 1992; 41(RR-14):1–7.
98. DHHS/PHS. Food standards: amendment of the standards of identity for enriched cereal-grain products to require the addition of folic acid: final rule (21 CFR Parts 136, 137 and 139). Fed Regist. 1996;61:8781–97.
99. DHHS/PHS. Food additives permitted for direct addition to food for human consumption: folic acid (folacin): final rule (21CFR Part 172). Fed Regist. 1996;61:8797–807.
100. Ray JG, Meier C, Vermeulen MJ, et al. Prevalence of trisomy 21 following folic acid food fortification. Am J Med Genet A. 2003;120A(3):309–13.
101. Collins JS, Olson RL, DuPont BR, et al. Prevalence of aneuploidies in South Carolina in the 1990s. Gen Med. 2002;4(3):131–5.
102. Canfield MA, Collins JS, Botto LD, et al. Changes in the birth prevalence of selected birth defects after grain fortification with folic acid in the United States: findings from a multi-state population-based study.

Birth Defects Res A Clin Mol Teratol. 2005; 73(10):679–89.
103. Simmons CJ, Mosley BS, Fulton-Bond CA, et al. Birth defects in Arkansas: is folic acid fortification making a difference? Birth Defects Res A Clin Mol Teratol. 2004;70(9):559–64.
104. Martin JA, Hamilton BE, Sutton PD, et al. Births: final data for 2002. Natl Vital Stat Rep. 2003; 52(10).
105. Martin JA, Hamilton BE, Sutton PD, et al. Births: final data for 2005. Natl Vital Stat Rep. 2007;56(6).
106. Czeizel AE, Puho E. Maternal use of nutritional supplements during the first month of pregnancy and decreased risk of Down's syndrome: case–control study. Nutrition. 2005;21(6):698–704; discussion 74.
107. Hollis N, Allen EG, Oliver TR, et al. Preconception folic acid supplementation and risk for chromosome 21 nondisjunction: a report from the National Down Syndrome Project. Am J Med Genet A. 2013; 161(3):438–44.
108. Warburton D. Biological aging and the etiology of aneuploidy. Cytogenet Genome Res. 2005;111(3–4): 266–72.
109. Ghosh S, Feingold E, Chakraborty S, et al. Telomere length is associated with types of chromosome 21 nondisjunction: a new insight into the maternal age effect on Down syndrome birth. Hum Genet. 2010; 127(4):403–9.
110. Hassold T, Chiu D. Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. Hum Genet. 1985;70(1):11–7.

Fertility Preservation for Cancer Patients

7

Suneeta Senapati and Clarisa R. Gracia

7.1 Introduction

Over 100,000 reproductive-aged women and men are diagnosed with cancer in the USA annually [1]. In females, the most common malignancies include cancer of the breast, cervix, uterus, thyroid, skin, and hematopoietic system. Testicular and hematologic malignancies most often affect reproductive age males. Cancers are now often treated with a combination of surgery, chemotherapy and radiotherapy. In addition to malignancies, there are several nonmalignant disorders that affect both females and males, which may require potentially gonadotoxic treatments including systemic lupus erythematosus, refractory sickle cell disease, and thalassemia [2–4].

Innovations in treatment regimens over time have led to longer survival in patients with these disorders with a resulting increased interest in long-term quality of life. One of the important quality of life issues that has emerged is the desire to have biological children. Therefore, more attention has been focused on the preservation of fertility and reproductive needs of cancer survivors [5]. Indeed, both the American Society of Reproductive Medicine and the American Society of Clinical Oncology have encouraged clinicians to educate their patient about the risks of cancer treatments and options for fertility preservation [6, 7]. Unfortunately, numerous barriers hamper the implementation of these recommendations, and there is a need to educate clinicians about the reproductive risks and fertility preservation options available for females and males facing fertility threatening treatments. This chapter will review the reproductive risks associated with cancer treatments, available fertility preservation techniques, safety of pregnancy, and family planning methods for reproductive age cancer patients.

7.2 Reproductive Risks Associated with Cancer and Cancer Treatments

Gonadal failure is well recognized as a possible sequela of cancer treatment in both sexes, as both chemotherapy and radiation therapy can impact reproductive potential as well as endocrine function. However, one of the challenges in studying the reproductive risks of cancer therapy is that outcomes such as fertility are difficult to study. In particular, prospective time to pregnancy studies are generally not possible when the exposure, cancer, is relatively rare. Therefore, most studies have assessed other related outcomes such as menstrual function, reported pregnancy after cancer treatment, measures of ovarian reserve, or semen parameters. Most of the information on reproductive risks for males and females stems epidemiologic data from the childhood cancer

S. Senapati, M.D. • C.R. Gracia, M.D., M.S.C.E. (✉)
Department of Obstetrics and Gynecology, University of Pennsylvania, 3701 Market Street Suite 800, Philadelphia, PA 19104, USA
e-mail: cgracia!@obgyn.upenn.edu

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_7,

Table 7.1 Risk of ovarian failure and chemotherapeutic regimens

High risk > 80 %	Intermediate risk	Low risk < 20 %	Minimal to no risk
Cyclophosphamide + methotrexate/epirubicin/doxorubicin + 5-fluorocuracil × 6 cycles in women ≥40 years (CMF, CEF, CAF)	Cyclophosphamide + methotrexate/epirubicin/doxorubicin + 5-fluorocuracil × 6 cycles in women 30–39 years (CMF, CEF, CAF)	Cyclophosphamide + methotrexate/epirubicin/doxorubicin + 5-fluorocuracil × 6 cycles in women <30 years (CMF, CEF, CAF)	Radioactive iodine
Pretreatment for hematopoetic stem cell transplant with cyclophosphamide/total body irradiation/busulfan	Doxorubicin + cyclophosphamide in women >40 years (AC)	Doxorubicin + cyclophosphamide in women <40 years (AC)	Vincristine
Protocols containing procarbazine (MOPP, MVPP, COPP, BEACOPP, MOPP/ABVD, COPP/ABVD, ChlVPP, ChlVPP/EVA		Adriamycin + Bleomycin + Vinblastine + Dacarbazine (ABVD)	Methotrexate
		Cyclophoshamide + Doxorubicin + oncovin + prednisone × 4–6 cycles (CHOP)	Fluorouracil
		Cyclophoshamide + oncovin + prednisone (COP)	
		Anthracycline + cytrabine	

Unknown risk: tyrosine kinase inhibitors, monoclonal antibodies, taxanes, oxaliplatin, irinotecan
Reprinted and modified Table 2 from Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006 Jun 20;24(18):2917–31

survivorship study (CCSS), a large retrospective cohort study of over 20,000 cancer survivors assessing a variety of late effects of childhood cancer.

7.2.1 Females

In women, accelerated gamete depletion related to chemotherapy and radiotherapy is nested in the dogma of a finite follicular pool. While the concept that the size of the follicular pool is defined in fetal life has recently been challenged [8], it is generally accepted that the pool of immature follicles peaks in size around 20 weeks of fetal life and is depleted through ovulation or atresia until menopause [9]. In women who undergo cancer treatments, atresia may be accelerated due to the gonadotoxic effects of certain treatment modalities. The effect on future fertility can be difficult to predict, as it is dependent upon a variety of factors including the dose and duration of chemotherapy or radiation exposure, the patient's age at time of exposure, genetics, and other factors (Table 7.1). Alkylating agents in particular, such as cyclophosphamide and busulfan, have a 3.98-fold increased age-adjusted risk of ovarian failure compared to other antineoplastic agents [10] and exert their gonadotoxic effects in a dose-dependent fashion [11, 12]. Combined chemotherapy regimens for breast cancer typically include cyclophosphamide, and the risk of amenorrhea is age dependent. For example, amenorrhea occurs in approximately 10 % of women <35, 40 % of women between 35 and 40 years of age, and 70 % of women over 40 treated with multi-agent chemotherapy for breast cancer [13]. Studies of ovarian reserve in menstruating women have shown that ovarian measures are impaired in cancer survivors' exposed to alkylating agents in a dose-dependent fashion [14].

Cranial, spinal, total body, abdominal, pelvic, and total body irradiation can damage the gonadal tissues, or the neuroendocrine pathways critical to normal reproductive function. In terms of targeted pelvic radiation, the degree of gonadotoxic

effect is both dose and age dependent. A mathematical model to predict age of ovarian failure according to radiation exposure suggested that the effective sterilizing dose needed for a 20-year-old patient is 16.5 Gray (Gy) and 14.3 Gy is sufficient for women at 30 years [15]. Recent data from the CCSS demonstrated that the relative risk of achieving pregnancy at least 5 years posttreatment in female childhood cancer survivors exposed to 5–10 Gy of radiation to the pelvis was 0.56 and dropped to 0.18 for exposure greater than 10 Gy compared to a similar-age sibling cohort [11]. Furthermore, patients receiving pelvic radiation should be counseled regarding the possible need for gestational surrogacy in the setting of premenarchal pelvic radiation greater than 1 Gy or postmenarchal pelvic radiation greater than 10 Gy as both have been associated with an increased risk of stillbirth or neonatal death [16].

7.2.2 Males

While the exact mechanism of damage to spermatogenesis from cancer therapies is unclear, both chemotherapy and radiotherapy can result in damage to the seminiferous tubules, including spermatogonial cells and Sertoli cells [17]. In males, chemotherapy also causes significant damage to testicular tissue in an agent-specific and dose-dependent manner. Alkylating agents have been shown to be the most toxic (Table 7.2). In the CCSS study, males with higher cumulative alkylator exposure were less likely to have fathered a child (HR 0.16 for an AAD score of 2 and a HR 0.16 for an AAD score of 6–11 [18]. Antimetabolite therapy such as methotrexate and mercaptopurine appear to have no effect on male fertility, while cisplatin-based regimens temporarily impair spermatogenesis with recovery shown in a significant number of patients [19]. The testes are exquisitely sensitive to radiotherapy with doses as low as 0.1 Gy resulting in temporary arrest of spermatogenesis (Pryant). Higher doses have been shown to cause azoospermia with doses of <1 Gy, 2–3 Gy, and 4–6 Gy, resulting in azoospermia lasting 30 months, 5 years, and permanently, respectively [20–23].

Table 7.2 Effects of cancer treatment on sperm production

Agent	Effect
Radiation (2.5 Gy to testis) Chlorambucil (1.4 g/m^2) Cyclophosphamide (19 g/m^2) Proocarbazine (4 g/m^2) Melphalan (140 mg/m^2) Cisplatin (500 mg/m^2)	Prolonged azoospermia
Carmustine (1 g/m^2) Lomustine (500 mg/m^2)	Azoospermia in adulthood with prepubertal treatment
Cyclophosphamide 10–19 g/m^2) Busulfan (600mg/m^2) Ifosfamide (42 g/m^2) Carmustine 300 mg/m^2) Nitrogen mustard Actinomycin D	Azoospermia likely
Carboplatin (2 g/m^2)	Prolonged azoospermia not often observed
Adriamycin (770 mg/m^2) Thiotepa (400 mg/m^2) Cytosine arbinoside (1 g/m^2) Vinblastine (50 g/m^2) Vincristine (8 g/m^2)	Temporary reductions alone; additive risk for prolonged azoospermia in combination with high risk agents
Amsacrine, bleomycin, dacarbazine, daunorubicin, epirubicin, etoposide, fludarabine, fluorouracil, 6-mercaptopurine, methotrexate, mitoxantrone, thioguanine	Temporary reductions alone; additive risk for prolonged azoospermia possible
Prednisone, interferon-α	Little to no risk of azoospermia

Unknown risk: tyrosine kinase inhibitors, monoclonal antibodies, taxanes, oxaliplatin, irinotecan

Reprinted and modified Table 1 from Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006 Jun 20;24(18):2917–31

7.2.3 Surgery

Surgical treatment for malignancy is another potential cause of infertility. In females, removal of the ovaries or uterus may be necessary to treat gynecologic malignancies. For males, orchiectomy is often recommended for the treatment of testicular cancers. In addition, pelvic surgery may alter the ability to conceive through natural ejaculation by damaging the neurologic or functional mechanisms of sperm delivery.

7.2.4 Cancer and Infertility

In addition to cancer treatments, there is speculation that the cancer disease process itself may impair fertility. In male patients, a cancer diagnosis is associated with decreased semen parameters and decreased fertility, particularly in patients with testicular malignancies and lymphoma [24–27]. Similarly, female patients with lymphoma have been noted to have lower estradiol levels during controlled ovarian stimulation even before gonadotoxic treatment suggesting possible impaired ovarian reserve [28]. In addition, patients with BRCA-1 mutations have been noted to have lower oocyte yield and a higher likelihood of poor response to control ovarian hyperstimulation than patients with BRCA-2 mutations or patients with breast cancer and unknown BRCA-mutation status [29]. While cancers themselves may be associated with some decreased fertility, the major effects on reproductive function are from the associated treatments. More data are needed to help predict the impact of cancer therapies on reproductive function. Prospective studies evaluating the predictive value of individual and treatment characteristics would be very useful to provide individualized counseling to patients regarding the reproductive risks and for targeting fertility preservation strategies to those at highest risk. For example, a recent study has demonstrated that pretreatment levels of AMH are predictive of posttreatment ovarian reserve in reproductive age women undergoing chemotherapy [30].

7.3 Approach to Fertility Preservation Consultation

In 2006 the American Society of Clinical Oncology released recommendations for fertility preservation in cancer patients encouraging oncologists to address the possibility of infertility with patients and either be prepared to discuss options or refer patients to reproductive specialists for fertility preservation counseling [6]. The consultation should include all available methods including those considered experimental, as well as those involving donor gametes, embryos, or adoption. This discussion must also take into consideration the disease process involved, the current health of the patient, and safety of pregnancy in the future. Some malignancies may present in acute crises such that fertility preservation options prior to gonadotoxic treatments may be limited. Those cases in which there is a window between diagnosis and treatment may allow patients to pursue a spectrum of options including gamete or embryo cryopreservation. Some patients may benefit from multiple modalities of fertility preservation techniques; for example, it may be feasible for a patient to pursue both oocyte cryopreservation and ovarian tissue cryopreservation, or embryo cryopreservation followed by gonadotropin releasing hormone analog treatment [31].

One of the challenges of the fertility preservation consultation is weighing the potential risks and benefits of fertility preservation strategies when treatment protocols may change over time. For example, sometimes the initial planned chemotherapy may be low risk for gonadal failure, but may become more gonadotoxic in the event of relapse. Thus, the options that are likely to be most successful may change over time depending upon the patient's disease status. Also, it is imperative to discuss the possibility that gametes, embryos, or tissues cryopreserved may never be utilized in the future. This situation may arise if the patient is able to conceive without assisted reproductive technologies, or if the patient dies. Thus, a discussion of disposition of any gametes, embryos, or gonadal tissue is a necessity. Moreover, some women may survive the disease process and treatment, but be unable to carry a pregnancy due to obstetric risks of treatment sequelae or their health status. The possible need for a gestational carrier should be addressed at the time of any fertility preservation treatment to ensure that the appropriate infectious disease testing and screening is performed so that tissue, gametes, or embryos may be safely used in the future.

The appropriate fertility preservation consultation should be timely and include a discussion of all techniques available (Table 7.3). Having an easily accessible point of contact for fertility preservation services such as a patient navigator can facilitate the efficiency of this process, as

many of these patients may have limited time between diagnosis and impending gonadotoxic treatment. In breast cancer patients, it has been shown that fertility preservation does not prolong the time interval between diagnosis and chemotherapy [32]. Thus, this consultation should not be viewed as an addition, but rather, a necessary component in the setting of a newly diagnosed malignancy or recurrence in a reproductive-aged patient. A multidisciplinary approach is critical, as the input of and collaboration between oncologists, radiation oncologists, urologists, genetic counselors, mental health professionals, patient navigators, and financial counselors are key to providing comprehensive care.

7.3.1 Fertility Preservation Options for Females

7.3.1.1 Embryo Cryopreservation

Embryo cryopreservation is one of the most established assisted reproductive technologies available to female cancer patients for fertility preservation. This technique typically requires controlled ovarian hyperstimulation with retrieval of mature oocytes followed by insemination of sperm from a committed partner or donor. Much of the data used to counsel these patients is currently derived from cryopreservation cycles of infertility populations and donor populations (Table 7.4). US national statistics from the Society for Assisted Reproductive Technologies indicate a live birth rate of 38.7 % per thawed embryo transfer for women less than 35 years of age. These rates decline with age and should be adjusted as per individual center-specific ongoing pregnancy and live birth data. Prospective data regarding ongoing pregnancy and live birth rates from embryos cryopreserved after cancer diagnosis and treatment are limited.

Table 7.3 Fertility preservation options

	Male	Female
Prepubertal	Germ cell extraction*	Ovarian tissue cryopreservation Ovarian transposition
Postpubertal	Sperm cryopreservation Surgical sperm extraction	Embryo cryopreservation Oocyte cryopreservation Gonadotropin releasing hormone analogues Ovarian transposition In vitro maturation Ovarian tissue cryopreservation

*Experimental

7.3.1.2 Mature Oocyte Cryopreservation

Mature oocyte cryopreservation may be an excellent option for postpubertal females without a committed partner who do not wish to use donor sperm. This technique is now considered an established technique by the American Society for Reproductive Medicine [33]. Oocyte cryopreservation was originally described using slow freezing techniques. However, oocyte vitrification has now emerged as the preferred technology with two randomized controlled trials suggesting improved oocyte survival with vitrification compared to slow freezing techniques (OR 2.46, 95 % CI [1.82–3.32]) [34–36]. The first live birth from slow frozen oocytes was described in 1986 and the first live birth from a vitrified oocyte in 1999 [37, 38]. Data on the success rates of oocyte cryopreservation are principally from egg donor/recipient cycles or infertile couples with supernumerary oocytes available. Four randomized controlled trials comparing outcomes with vitrified and fresh oocytes in IVF/ICSI cycles demonstrate

Table 7.4 Age-stratified live birth rates per embryo transfer, SART 2010

	Oocyte donors	<35 years	35–37 years	38–40 years	41–42 years	>42 years
Fresh cycle: Live birth/ET	55.6	47.8	38.4	28.1	16.8	6.3
Thawed: Live birth/ET	34.8	38.7	35.1	28.5	21.4	15.3
Ave No. ET	2.0	1.9	1.9	2.1	2.2	2.1

Based on 146,693 cycles

that oocyte survival after vitrification and warming ranged between 90 and 97 %, fertilization rates were between 71 and 79 %, implantation rates were 17–41 %, and clinical pregnancy rates per transfer ranged from 36 to 61 % [39–41]. Indeed, with the improvement of laboratory techniques, oocyte cryopreservation success approaches the success of fresh IVF in some clinics. A meta-analysis including three randomized controlled trials of fresh and vitrified oocytes indicated that fertilization rates, cleavage rates, and ongoing pregnancy rates are comparable [35, 42].

7.3.1.3 Controlled Ovarian Hyperstimulation in Cancer Patients

Selecting a protocol for controlled ovarian hyperstimulation for either mature oocyte or embryo cryopreservation in cancer patients is challenging, as response can be highly variable. There is conflicting data regarding the degree of difference in response to stimulation in women with malignancy compared to the general population. A meta-analysis of seven retrospective studies on the ovarian response in cancer patients to age-matched healthy controls suggested that women with cancer have a significantly lower oocyte yield but with comparable fertilization rates [43].

Most ovarian hyperstimulation protocols were established for infertile populations or oocyte donors. However, the response in these populations may not be predictive of patients pursuing fertility preservation since many of them have untested fertility. While some patients may have poor oocyte yield due to their age, disease state, and possible underlying infertility, others may have a very robust response. Therefore, the use of antagonist protocols provide flexibility and are safer since gonadotropin releasing hormone agonists may be used to induce oocyte maturation in order to prevent ovarian hyperstimulation syndrome [44].

There is a theoretical concern regarding the impact of elevated estradiol levels during ovarian stimulation in hormonally responsive malignancies. As such, protocols aimed at reducing the estradiol levels with aromatase inhibitors have been suggested to minimize this potential risk [45, 46]. These protocols have been shown to result in acceptable oocyte and embryo yields.

Data are limited in patients who present outside of the early follicular phase and whose treatment must be expedited. However, several small studies suggest that stimulation with a GnRH antagonist in conjunction with exogenous gonadotropins results in acceptable numbers of oocyte/embryo to cryopreserve [47, 48].

7.3.1.4 In Vitro Oocyte Maturation

In vitro maturation involves the retrieval of immature oocytes with minimal or no ovarian stimulation and maturation of oocytes in the laboratory. For the purpose of fertility preservation, mature oocytes may then be cryopreserved or fertilized for embryo cryopreservation. This strategy may be particularly attractive in situations where cancer therapy must be initiated urgently. While live births have been reported with this technique in the setting of polycystic ovarian syndrome [49], live births are estimated to be lower than with traditional ovarian stimulation [50, 51]. There are limited data about the success of this strategy in cancer patients. This option may also be used in the setting of oocytes retrieved from ovarian biopsy for tissue cryopreservation although, similarly, obstetric outcome data is currently unavailable. This methodology remains an important area of research in fertility preservation.

7.3.1.5 Ovarian Tissue Cryopreservation and Transplantation

Ovarian tissue cryopreservation involves removal of the whole ovary or a portion of the ovarian cortical tissue, typically via laparoscopy, followed by cryopreservation with the intent of orthotopic or heterotopic autologous transplantation in the future. The process involves isolating the follicle-rich ovarian cortex and cutting it into 1 mm thick fragments, and cryopreservation either by vitrification or slow-freeze techniques [52]. Unlike embryo and oocyte cryopreservation, which typically require a 2 week period for controlled ovarian hyperstimulation, ovarian tissue can be removed and cryopreserved without gonadotropin or other hormonal preparation. Therefore, it is a strategy typically used for

females who need urgent therapy. In addition, it is the only technique available for fertility preservation in prepubescent females whose ovaries do not respond to gonadotropin stimulation. As the success of this method of fertility preservation is dependent upon an adequate follicular pool prior to tissue biopsy, it is not recommended in women over 40 years old [53]. At the time of this publication, 22 healthy live births have been reported after orthotopic tissue transplantation with a wide range of conditions including lymphoma, breast cancer, Ewing sarcoma, neuroectodermic tumor, microscopic polyangitis, thalassemia, sickle disease, and idiopathic premature ovarian failure [54–65]. The transplant viability typically ranges from 60 days to 3 years; thus, patients interested in this option should undergo transplantation when they are ready to conceive [65]. Care should be taken to counsel patients with hematologic malignancies as to the theoretical risk of reseeding malignant cells with transplantation of ovarian tissue [66–70]. In addition, transplantation is not recommended in other situations where cancer cells may be present in ovarian tissue. Alternative methods of utilizing ovarian tissue, including oocytes/follicular maturation in vivo to achieve a pregnancy, has not been successful to date. While still considered experimental, ovarian tissue cryopreservation remains a promising option for both prepubertal and reproductive-aged female patients desiring future fertility.

7.3.1.6 Ovarian Transposition

In the setting of anticipated pelvic irradiation, ovarian transposition, or oophoropexy, may be appropriate. This involves surgically moving the ovaries away from the path of radiation, typically to the pelvic sidewall or into the posterior cul de sac. This was first described in a case series of women planning for pelvic irradiation for Hodgkin's Disease [71] and has since been used for pelvic malignancies, medulloblastoma and other cases that require pelvic irradiation [72, 73]. In cases in which the uterus or uterine vasculature has been damaged and patients are unable to carry a pregnancy, patients may be able to pursue in vitro fertilization with a gestational carrier.

7.3.1.7 Gonadotropin Hormone Releasing Analogues

Ovarian suppression during chemotherapy with gonadotropin releasing hormone (GnRH) agonists or GnRH antagonists may also be an option for patients who do not have the desire or an adequate time window for gamete/embryo cryopreservation. The theoretical effectiveness of this strategy is extrapolated from the observation that prepubertal cancer patients who are exposed to gonadotoxic therapy are less likely to have impairment of gonadal function [74]. Thus, it is hypothesized that temporary suppression of the pituitary–gonadal axis, decreased gonadal perfusion, and possibly a direct gonadal effect may reduce germ cell apoptosis. Moreover, these agents may confer an added benefit of prevention of menorrhagia, particularly in patients who develop severe thrombocytopenia from the myelosuppressive effects of chemotherapy [75]. Studies of the efficacy of GnRH analogues in preserving fertility have had mixed results. Two separate meta-analyses of the use of gonadotropin releasing hormone analogues for downregulation administered prior to start of gonadotoxic treatment suggest a benefit with respect to resumption of menses after chemotherapy and ovulation, but no difference in pregnancy rates or live birth rates [76, 77]. Additional studies are needed to evaluate whether GnRH agonists are associated with improved long-term fertility.

7.3.2 Male Fertility Preservation Techniques

7.3.2.1 Sperm Cryopreservation

Sperm cryopreservation remains the standard method for fertility preservation in postpubertal males. Sperm is typically collected by masturbation prior to the initiation of chemotherapy or radiation therapy. Depending on the semen analysis, multiple samples may be collected, which can be used for intrauterine insemination or IVF in the future. Patients with ejaculatory dysfunction may benefit from phosphodiesterase inhibitors, vibratory stimulation, or electroejaculation in order to obtain adequate samples for cryopreservation [78, 79].

7.3.2.2 Surgical Sperm Extraction

Surgical sperm extraction may also be an option for those patients from whom an ejaculated specimen cannot be obtained or for those with azoospermia. These techniques include percutaneous epididymal sperm aspiration (PESA), testicular sperm extraction (TESE), testicular sperm aspiration (TESA), and microsurgical epididymal sperm aspiration (MESA). In the setting of surgical sperm extraction, or severe abnormalities in ejaculated sperm, the patient should be counseled that using that sperm in the future will require in vitro fertilization with intracytoplasmic sperm injection. Isolation of germ cells from testicular tissue biopsy has also been studied and may be an option for prepubertal male cancer patients [80, 81]. At this time, there is no data on human transplantation of these germ cells and thus this remains a purely investigational technique.

7.4 Pregnancy and Survivorship Care for Cancer Patients

7.4.1 Contraception

A discussion of contraceptive needs for cancer patients is often overlooked in the setting of a new cancer diagnosis and is a critical component of a fertility preservation consultation. While a patient's anxiety and distress regarding the prospect of infertility may be substantial, an unintended pregnancy in the setting of a cancer diagnosis can be emotionally devastating and could place the female patient at considerable obstetric-related risk. While population-based data regarding the incidence of unintended pregnancy in the USA are currently unavailable, case–control studies in US and Danish populations suggest that cancer survivors are more likely to terminate a pregnancy compared to controls [82, 83].

Cancer survivors present unique challenges for family planning. Cancer diagnosis including hormone receptivity, history of thromboembolic disease, liver dysfunction, hypertension, and other comorbidities must all be considered in recommendations for contraceptive therapy. The Society for Family Planning issued contraception guidelines in 2012 for patients with cancer that included avoiding combination estrogen–progestin therapy for active cancer or those treated with the past 6 months, encouraging copper-containing intrauterine device use in patients with breast cancer and consider levonorgestrel-containing intrauterine systems for patients with anemia to minimize menstrual blood loss [84]. Issues for which evidence remains limited include combined estrogen–progestin therapy for patients with prior chest radiation, levonorgestrel-containing intrauterine devices for breast cancer patients on tamoxifen, optimal contraception in cancer survivors with osteopenia or osteoporosis, and emergency contraception use in patients with breast cancer who decline other methods.

7.4.2 Preconception Counseling

Preconception counseling for cancer survivors should include age-appropriate recommendations regarding prenatal screening, immunization, and genetic and nutrition counseling. Additionally, cancer diagnosis, stage, prognosis, cumulative chemotherapy and/or radiation exposures, and surveillance needs must all be considered in determining the expected obstetric risks. Patients who are known or suspected to have developed a malignancy as a result of a genetic or hereditary syndrome may benefit from meeting with a genetics specialist. Preimplantation genetic diagnosis and prenatal diagnosis should be discussed as options.

Prior exposure to anthracycline agents such as doxorubicin and daunorubicin is associated with left ventricular dysfunction in a dose dependent fashion [85]. Thus, the Children's Oncology Group Long-Term Follow-up Guidelines for Survivors of Childhood, Adolescent, and Young Adult Cancer has recommended that women who have received a cumulative anthracycline dose of 300 mg/m^2 or greater, who have received 30 Gy or more of radiation directed towards the thoracic tissues, or who have received the combination of any dose of radiation to the thoracic tissues with chemotherapy including anthracyclines at any dose or high doses of cyclophosphamide should have preconception cardiac evaluation [86].

While the overall incidence of peripartum cardiomyopathy is suggested to be low in retrospective cohort studies of childhood cancer survivors [87], these patients should be managed in collaboration with a high risk obstetrics team and a cardiology team.

Patients who have had prior exposure to bleomycin, carmustine, or thoracic radiation, are a risk for pneumonitis [85, 88]. The rates of chronic pulmonary disease after these exposure range from 1 to 15 %. While there are no specific recommendations regarding preconception or prenatal monitoring in these patients, baseline pulmonary function testing should be considered.

In addition, exposures to chemotherapy, glucocorticoids, and radiation may damage other organ systems subclinically. The physiologic changes of pregnancy may unmask organ dysfunction and lead to significant pregnancy complications. In addition, cancer treatments can put patients at risk for a secondary malignancy during pregnancy. Thus, it is imperative that patients considering pregnancy be up to date regarding their surveillance with respect to recurrence, secondary malignancies, and late effects of prior treatment that could impact obstetric management.

7.4.3 Obstetric Outcomes in Cancer Survivors

Many patients and practitioners will have concerns about the risks of recurrence associated with pregnancy, and possible adverse obstetric and neonatal outcomes due to prior cancer treatments. Unassisted pregnancy after breast cancer is not associated with an increased risk of recurrence or reduction in survival [89], and data from childhood cancer survivors remote from therapy indicate that while women exposed to pelvic irradiation had a higher incidence of small for gestational age infants, there were no other differences in adverse obstetric outcomes compared to healthy controls [82]. There is little known about pregnancy outcomes after recent gonadotoxic exposure, the optimal duration of time from chemotherapy exposure to conception to minimize these risks. However, animal studies suggest that miscarriage and rates of birth defects are higher in mice that conceive during chemotherapy exposure [12] but the optimal duration of time from chemotherapy exposure to conception to minimize these risks is unknown. In addition, childhood cancer survivors remote from treatment do not appear to have an increased risk of congenital anomalies in their offspring [90]. It is generally recommended to wait 6 months to 1 year to conceive after a cancer diagnosis.

7.4.4 Additional Survivorship Care

For patients who have experienced acute or premature ovarian failure, their counseling should include a discussion of the effects of a hypoestrogenic state and options for management. Some of these patients will experience menopausal symptoms transiently during treatment that will resolve with the resumption of menses, while others may continue to have hot flashes, night sweats, and vaginal dryness for years after treatment. There are no guidelines with respect to recommendations for hormone replacement therapy in this patient population. However, due to clear differences with respect to age and risk modifiers, data from large population studies such as the Women's Health Initiative are unfortunately not generalizable to this population. Certainly, there are concerns with respect to the risks of recurrence in hormonally responsive malignancies as well as the risk of thromboembolic events. Nonetheless, in nonhormonally sensitive tumors, the benefits of hormone replacement therapy outweigh the risks in reproductive-aged women. In addition to hormone replacement therapy, selective serotonin reuptake inhibitors, venlaxafine, and gabapentin, and lifestyle modification may also be recommended to management of vasomotor symptoms [91]. Vaginal estrogen and lubricants may be effective for dyspareunia related to vaginal atrophy and appears to be safe in patients who are not eligible for systemic estrogen or combined hormonal therapy [92]. Additionally, patients with premature gonadal failure will be at risk of developing osteoporosis. While there are no data with respect to incidence and optimal treatment in this patient

population, calcium and vitamin D supplementation, weight bearing exercises, and bisphosphonate therapy may all be considered; bisphosphonate therapy should be reserved for those who have no plans for childbearing in the future. Hormone replacement therapy has been suggested to increase bone mineral density in female patients after bone marrow transplantation with minimal risk [93]. As with therapy for menopausal symptoms, the risks and benefits should be discussed with the patient and decisions should be made in collaboration with the oncology team.

Finally, both male and female cancer survivors may experience sexual dysfunction. While all phases of the sexual response cycle may be impacted, in particular men are more likely to experience erectile dysfunction, and women are more likely to experience decreased libido and vaginal dryness [94]. Counseling regarding cancer-specific sexual issues as well as sexual rehabilitation has been suggested to decrease psychological distress and improve quality of life in cancer patients [95, 96]. While the study of sexual function in this patient population continues to evolve, at a minimum, ascertainment of sexual dysfunction is important to survivorship care.

7.5 Conclusion

The management of reproductive issues in the setting of a malignancy is complex and requires diligence from all providers involved. Clinicians should discuss the reproductive risks, fertility preservation methods, and contraceptive needs of patients at the time of diagnosis in order to maximize the reproductive health and options for having a family in the future. With increasing awareness and ongoing research in the area of Oncofertility, we hope that this field will continue to advance to meet the needs of this unique patient population.

References

1. Howlader N, Noone AM, Krapcho M, Neyman N, Aminou R, Altekruse SF, Kosary CL, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Chen HS, Feuer EJ, Cronin KA, editors. SEER Cancer Statistics Review. Bethesda, MD: National Cancer Institute; 2009. Available from http://seer.cancer.gov/csr/1975_2009_pops09/.
2. Bhatia M, Walters MC. Hematopoietic cell transplantation for thalassemia and sickle cell disease: past, present and future. Bone Marrow Transplant. 2008; 41(2):109–17.
3. Henes M, Henes JC, Neunhoeffer E, et al. Fertility preservation methods in young women with systemic lupus erythematosus prior to cytotoxic therapy: experiences from the FertiPROTEKT network. Lupus. 2012;21(9):953–8.
4. Hsieh MM, Kang EM, Fitzhugh CD, et al. Allogeneic hematopoietic stem-cell transplantation for sickle cell disease. N Engl J Med. 2009;361(24):2309–17.
5. Letourneau JM, Ebbel EE, Katz PP, et al. Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer. Cancer. 2012;118(6):1710–7.
6. Lee SJ, Schover LR, Partridge AH, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006;24(18):2917–31.
7. Practice Committee of American Society for Reproductive Medicine: Practice Committee for Society of Assisted Reproductive Technology. Ovarian tissue and oocyte cryopreservation. Fertil Steril. 2008;90(5 Suppl):S241–6.
8. Johnson J, Bagley J, Skaznik-Wikiel M, et al. Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. Cell. 2005;122(2):303–15.
9. Strauss JF, Barbieri RL. Yen and Jaffe's reproductive endocrinology: physiology, pathophysiology, and clinical management. 6th ed. Philadelphia, PA: Saunders Elsevier; 2009.
10. Meirow D. Reproduction post-chemotherapy in young cancer patients. Mol Cell Endocrinol. 2000;169(1–2): 123–31.
11. Green DM, Kawashima T, Stovall M, et al. Fertility of female survivors of childhood cancer: a report from the childhood cancer survivor study. J Clin Oncol. 2009;27(16):2677–85.
12. Meirow D, Epstein M, Lewis H, et al. Administration of cyclophosphamide at different stages of follicular maturation in mice: effects on reproductive performance and fetal malformations. Hum Reprod. 2001;16(4):632–7.
13. Petrek JA, Naughton MJ, Case LD, et al. Incidence, time course, and determinants of menstrual bleeding after breast cancer treatment: a prospective study. J Clin Oncol. 2006;24(7):1045–51.
14. Gracia CR, Sammel MD, Freeman E, et al. Impact of cancer therapies on ovarian reserve. Fertil Steril. 2012;97(1):134–40.e1.
15. Wallace WH, Thomson AB, Saran F, et al. Predicting age of ovarian failure after radiation to a field that includes the ovaries. Int J Radiat Oncol Biol Phys. 2005;62(3):738–44.

16. Signorello LB, Mulvihill JJ, Green DM, et al. Stillbirth and neonatal death in relation to radiation exposure before conception: a retrospective cohort study. Lancet. 2010;376(9741):624–30.
17. Meistrich ML, Finch M, da Cunha MF, et al. Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. Cancer Res. 1982;42(1):122–31.
18. Green DM, Kawashima T, Stovall M, et al. Fertility of male survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. J Clin Oncol. 2010;28(2):332–9.
19. Hansen PV, Hansen SW. Gonadal function in men with testicular germ cell cancer: the influence of cisplatin-based chemotherapy. Eur Urol. 1993;23(1):153–6.
20. Pryzant RM, Meistrich ML, Wilson G, et al. Long-term reduction in sperm count after chemotherapy with and without radiation therapy for non-Hodgkin's lymphomas. J Clin Oncol. 1993;11(2):239–47.
21. Sandeman TF. The effects of x irradiation on male human fertility. Br J Radiol. 1966;39(468):901–7.
22. Hahn EW, Feingold SM, Simpson L, et al. Recovery from aspermia induced by low-dose radiation in seminoma patients. Cancer. 1982;50(2):337–40.
23. Centola GM, Keller JW, Henzler M, et al. Effect of low-dose testicular irradiation on sperm count and fertility in patients with testicular seminoma. J Androl. 1994;15(6):608–13.
24. Berthelsen JG, Skakkebaek NE. Gonadal function in men with testis cancer. Fertil Steril. 1983;39(1):68–75.
25. Sanger WG, Armitage JO, Schmidt MA. Feasibility of semen cryopreservation in patients with malignant disease. JAMA. 1980;244(8):789–90.
26. Spermon JR, Ramos L, Wetzels AM, et al. Sperm integrity pre- and post-chemotherapy in men with testicular germ cell cancer. Hum Reprod. 2006;21(7):1781–6.
27. Viviani S, Ragni G, Santoro A, et al. Testicular dysfunction in Hodgkin's disease before and after treatment. Eur J Cancer. 1991;27(11):1389–92.
28. Domingo J, Guillen V, Ayllon Y, et al. Ovarian response to controlled ovarian hyperstimulation in cancer patients is diminished even before oncological treatment. Fertil Steril. 2012;97(4):930–4.
29. Oktay K, Kim JY, Barad D, et al. Association of BRCA1 mutations with occult primary ovarian insufficiency: a possible explanation for the link between infertility and breast/ovarian cancer risks. J Clin Oncol. 2010;28(2):240–4.
30. Dillon KE, Sammel MD, Prewitt M, et al. Pretreatment antimullerian hormone levels determine rate of post-therapy ovarian reserve recovery: acute changes in ovarian reserve during and after chemotherapy. Fertil Steril. 2013;99(2):477–83.e1.
31. von Wolff M, Montag M, Dittrich R, et al. Fertility preservation in women – a practical guide to preservation techniques and therapeutic strategies in breast cancer, Hodgkin's lymphoma and borderline ovarian tumours by the fertility preservation network FertiPROTEKT. Arch Gynecol Obstet. 2011;284(2):427–35.
32. Baynosa J, Westphal LM, Madrigrano A, et al. Timing of breast cancer treatments with oocyte retrieval and embryo cryopreservation. J Am Coll Surg. 2009;209(5):603–7.
33. Fertility Experts Issue New Report on Egg Freezing; ASRM Lifts Experimental Label from Technique. 2012.
34. Cao YX, Chian RC. Fertility preservation with immature and in vitro matured oocytes. Semin Reprod Med. 2009;27(6):456–64.
35. Cobo A, Diaz C. Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril. 2011;96(2):277–85.
36. Smith GD, Serafini PC, Fioravanti J, et al. Prospective randomized comparison of human oocyte cryopreservation with slow-rate freezing or vitrification. Fertil Steril. 2010;94(6):2088–95.
37. Chen C. Pregnancy after human oocyte cryopreservation. Lancet. 1986;1(8486):884–6.
38. Kuleshova L, Gianaroli L, Magli C, et al. Birth following vitrification of a small number of human oocytes: case report. Hum Reprod. 1999;14(12):3077–9.
39. Cobo A, Kuwayama M, Perez S, et al. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. Fertil Steril. 2008;89(6):1657–64.
40. Parmegiani L, Cognigni GE, Bernardi S, et al. Efficiency of aseptic open vitrification and hermetical cryostorage of human oocytes. Reprod Biomed Online. 2011;23(4):505–12.
41. Rienzi L, Romano S, Albricci L, et al. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum Reprod. 2010;25(1):66–73.
42. Rienzi L, Cobo A, Paffoni A, et al. Consistent and predictable delivery rates after oocyte vitrification: an observational longitudinal cohort multicentric study. Hum Reprod. 2012;27(6):1606–12.
43. Friedler S, Koc O, Gidoni Y, et al. Ovarian response to stimulation for fertility preservation in women with malignant disease: a systematic review and meta-analysis. Fertil Steril. 2012;97(1):125–33.
44. Acevedo B, Gomez-Palomares JL, Ricciarelli E, et al. Triggering ovulation with gonadotropin-releasing hormone agonists does not compromise embryo implantation rates. Fertil Steril. 2006;86(6):1682–7.
45. Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. J Clin Oncol. 2008;26(16):2630–5.
46. Oktay K, Hourvitz A, Sahin G, et al. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. J Clin Endocrinol Metab. 2006;91(10):3885–90.
47. Nayak SR, Wakim AN. Random-start gonadotropin-releasing hormone (GnRH) antagonist-treated cycles

with GnRH agonist trigger for fertility preservation. Fertil Steril. 2011;96(1):e51–4.
48. von Wolff M, Thaler CJ, Frambach T, et al. Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase. Fertil Steril. 2009;92(4):1360–5.
49. Chian RC, Gilbert L, Huang JY, et al. Live birth after vitrification of in vitro matured human oocytes. Fertil Steril. 2009;91(2):372–6.
50. Benkhalifa M, Demirol A, Menezo Y, et al. Natural cycle IVF and oocyte in-vitro maturation in polycystic ovary syndrome: a collaborative prospective study. Reprod Biomed Online. 2009;18(1):29–36.
51. Fadini R, Dal Canto MB, Mignini Renzini M, et al. Effect of different gonadotrophin priming on IVM of oocytes from women with normal ovaries: a prospective randomized study. Reprod Biomed Online. 2009; 19(3):343–51.
52. Andersen CY, Kristensen SG, Greve T, et al. Cryopreservation of ovarian tissue for fertility preservation in young female oncological patients. Future Oncol. 2012;8(5):595–608.
53. Oktay K. Evidence for limiting ovarian tissue harvesting for the purpose of transplantation to women younger than 40 years of age. J Clin Endocrinol Metab. 2002;87(4):1907–8.
54. Andersen CY, Rosendahl M, Byskov AG, et al. Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. Hum Reprod. 2008;23(10):2266–72.
55. Dittrich R, Lotz L, Keck G, et al. Live birth after ovarian tissue autotransplantation following overnight transportation before cryopreservation. Fertil Steril. 2012;97(2):387–90.
56. Donnez J, Dolmans MM, Demylle D, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004;364(9443):1405–10.
57. Donnez J, Jadoul P, Pirard C, et al. Live birth after transplantation of frozen-thawed ovarian tissue after bilateral oophorectomy for benign disease. Fertil Steril. 2012;98(3):720–5.
58. Donnez J, Silber S, Andersen CY, et al. Children born after autotransplantation of cryopreserved ovarian tissue. a review of 13 live births. Ann Med. 2011;43(6): 437–50.
59. Donnez J, Squifflet J, Jadoul P, et al. Pregnancy and live birth after autotransplantation of frozen-thawed ovarian tissue in a patient with metastatic disease undergoing chemotherapy and hematopoietic stem cell transplantation. Fertil Steril. 2011;95(5):1787.e1–4.
60. Ernst E, Bergholdt S, Jorgensen JS, et al. The first woman to give birth to two children following transplantation of frozen/thawed ovarian tissue. Hum Reprod. 2010;25(5):1280–1.
61. Meirow D, Levron J, Eldar-Geva T, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med. 2005;353(3):318–21.
62. Revelli A, Marchino G, Dolfin E, et al. Live birth after orthotopic grafting of autologous cryopreserved ovarian tissue and spontaneous conception in Italy. Fertil Steril. 2013;99(1):227–30.
63. Roux C, Amiot C, Agnani G, et al. Live birth after ovarian tissue autograft in a patient with sickle cell disease treated by allogeneic bone marrow transplantation. Fertil Steril. 2010;93(7):2413.e15–9.
64. Sanchez-Serrano M, Crespo J, Mirabet V, et al. Twins born after transplantation of ovarian cortical tissue and oocyte vitrification. Fertil Steril. 2010;93(1):268.e11–3.
65. Silber S, Kagawa N, Kuwayama M, et al. Duration of fertility after fresh and frozen ovary transplantation. Fertil Steril. 2010;94(6):2191–6.
66. Dolmans MM, Marinescu C, Saussoy P, et al. Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe. Blood. 2010;116(16):2908–14.
67. Greve T, Clasen-Linde E, Andersen MT, et al. Cryopreserved ovarian cortex from patients with leukemia in complete remission contains no apparent viable malignant cells. Blood. 2012;120(22): 4311–6.
68. Salle B, Demirci B, Franck M, et al. Long-term follow-up of cryopreserved hemi-ovary autografts in ewes: pregnancies, births, and histologic assessment. Fertil Steril. 2003;80(1):172–7.
69. Schmidt KT, Rosendahl M, Ernst E, et al. Autotransplantation of cryopreserved ovarian tissue in 12 women with chemotherapy-induced premature ovarian failure: the Danish experience. Fertil Steril. 2011;95(2):695–701.
70. Shaw JM, Bowles J, Koopman P, et al. Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit the cancer to graft recipients. Hum Reprod. 1996;11(8):1668–73.
71. Le Floch O, Donaldson SS, Kaplan HS. Pregnancy following oophoropexy and total nodal irradiation in women with Hodgkin's disease. Cancer. 1976;38(6): 2263–8.
72. Lee CL, Lai YM, Soong YK, et al. Laparoscopic ovariopexy before irradiation for medulloblastoma. Hum Reprod. 1995;10(2):372–4.
73. Morice P, Castaigne D, Haie-Meder C, et al. Laparoscopic ovarian transposition for pelvic malignancies: indications and functional outcomes. Fertil Steril. 1998;70(5):956–60.
74. Wallace WH, Shalet SM, Tetlow LJ, et al. Ovarian function following the treatment of childhood acute lymphoblastic leukaemia. Med Pediatr Oncol. 1993; 21(5):333–9.
75. Meirow D, Rabinovici J, Katz D, et al. Prevention of severe menorrhagia in oncology patients with treatment-induced thrombocytopenia by luteinizing hormone-releasing hormone agonist and depomedroxyprogesterone acetate. Cancer. 2006;107(7): 1634–41.
76. Bedaiwy MA, Abou-Setta AM, Desai N, et al. Gonadotropin-releasing hormone analog cotreatment for preservation of ovarian function during gonadotoxic chemotherapy: a systematic review and meta-analysis. Fertil Steril. 2011;95(3):906-141.e1–4.

77. Chen H, Li J, Cui T, et al. Adjuvant gonadotropin-releasing hormone analogues for the prevention of chemotherapy induced premature ovarian failure in premenopausal women. Cochrane Database Syst Rev. (11):CD008018.
78. Ohl DA, Wolf LJ, Menge AC, et al. Electroejaculation and assisted reproductive technologies in the treatment of anejaculatory infertility. Fertil Steril. 2001;76(6):1249–55.
79. Tur-Kaspa I, Segal S, Moffa F, et al. Viagra for temporary erectile dysfunction during treatments with assisted reproductive technologies. Hum Reprod. 1999;14(7):1783–4.
80. Brinster RL. Male germline stem cells: from mice to men. Science. 2007;316(5823):404–5.
81. Brook PF, Radford JA, Shalet SM, et al. Isolation of germ cells from human testicular tissue for low temperature storage and autotransplantation. Fertil Steril. 2001;75(2):269–74.
82. Green DM, Whitton JA, Stovall M, et al. Pregnancy outcome of female survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. Am J Obstet Gynecol. 2002;187(4):1070–80.
83. Winther JF, Boice Jr JD, Svendsen AL, et al. Induced abortions in Danish cancer survivors: a population-based cohort study. J Natl Cancer Inst. 2009;101(9):687–9.
84. Patel A, Schwarz B. Cancer and contraception. Release date May 2012. SFP Guideline #20121. Contraception. 2012;86(3):191–8.
85. Carver JR, Shapiro CL, Ng A, et al. American Society of Clinical Oncology clinical evidence review on the ongoing care of adult cancer survivors: cardiac and pulmonary late effects. J Clin Oncol. 2007;25(25):3991–4008.
86. Group CsO. Healthy Living after Treatment for Childhood Cancer. 2008 [December 10, 2012]. Available from http://survivorshipguidelines.org/pdf/HeartHealth.pdf.
87. van Dalen EC, van der Pal HJ, van den Bos C, et al. Clinical heart failure during pregnancy and delivery in a cohort of female childhood cancer survivors treated with anthracyclines. Eur J Cancer. 2006;42(15):2549–53.
88. O'Sullivan JM, Huddart RA, Norman AR, et al. Predicting the risk of bleomycin lung toxicity in patients with germ-cell tumours. Ann Oncol. 2003;14(1):91–6.
89. Blakely LJ, Buzdar AU, Lozada JA, et al. Effects of pregnancy after treatment for breast carcinoma on survival and risk of recurrence. Cancer. 2004;100(3):465–9.
90. Green DM, Sklar CA, Boice Jr JD, et al. Ovarian failure and reproductive outcomes after childhood cancer treatment: results from the Childhood Cancer Survivor Study. J Clin Oncol. 2009;27(14):2374–81.
91. Schover LR. Premature ovarian failure and its consequences: vasomotor symptoms, sexuality, and fertility. J Clin Oncol. 2008;26(5):753–8.
92. Dew JE, Wren BG, Eden JA. A cohort study of topical vaginal estrogen therapy in women previously treated for breast cancer. Climacteric. 2003;6(1):45–52.
93. Castelo-Branco C, Rovira M, Pons F, et al. The effect of hormone replacement therapy on bone mass in patients with ovarian failure due to bone marrow transplantation. Maturitas. 1996;23(3):307–12.
94. Gracia CR. Reproductive health after cancer. Cancer Treat Res. 2010;156:3–9.
95. Canada AL, Schover LR, Li Y. A pilot intervention to enhance psychosexual development in adolescents and young adults with cancer. Pediatr Blood Cancer. 2007;49(6):824–8.
96. Neese LE, Schover LR, Klein EA, et al. Finding help for sexual problems after prostate cancer treatment: a phone survey of men's and women's perspectives. Psychooncology. 2003;12(5):463–73.

8 Reproductive Surgery and Computer-Assisted Laparoscopy: The New Age of Subspecialty Surgery Is Here

Shane T. Lipskind and Antonio R. Gargiulo

8.1 Computer-Assisted Reproductive Surgery: A Vision Fulfilled

Professor Ricardo Aziz, in his heartfelt Fertility and Sterility editorial of 2002, addressed the delicate topic of the role of reproductive surgeons in the age of in vitro fertilization [1]. It was a somber commentary that identified two main factors responsible for the epochal shift of many fertility specialists away from the surgical arena: on one side, the development of highly effective assisted reproductive technologies that rendered most tubal microsurgery obsolete; on the other, the success of advanced minimally invasive surgery, which changed the parameters by which the quality of reproductive surgery would be defined. However, the editorial's appeal to reproductive endocrinologists to continue to take responsibility for their patients' surgical needs was loud and clear: "*The American Society for Reproductive Medicine (ASRM) and the Society of Reproductive Surgeons (SRS) should not be timid in asserting their position as the homes of the world's finest reproductive surgeons. The efforts of the SRS to establish itself as the custodian of quality reproductive-organ surgery in women and men fits well with the very successful public campaign regarding 'prevention of infertility,' currently being undertaken by the ASRM. Reproductive surgeons and the SRS not only should serve as experts in the treatment of pelvic-factor infertility but should and will begin to take an activist and front-line role in improving the surgical care of men and women everywhere.*" To this end, one of the initiatives of the SRS was to partner with the American Association for Gynecologic Laparoscopists (AAGL) to sponsor the creation of postgraduate training opportunities in minimally invasive gynecologic surgery with a standardized minimal curriculum and a requirement for research. The AAGL/SRS Fellowship in Minimally Invasive Gynecologic Surgery initiative, inaugurated in 2001, has thrived over the past decade to graduate over 150 preceptees who have contributed in many ways to the advancement of minimally invasive surgery in this country and abroad. Thanks to such bold academic catalysts, general gynecologists with excellent technical skills in minimally invasive surgery are now present in most urban areas of this country, and access to this superior level of surgical care has improved. A regrettable shortcoming of this overall positive development is that the focus of most of these preceptorships has remained outside of the

S.T. Lipskind, M.D.
Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School, Boston, MA, USA

A.R. Gargiulo, M.D. (✉)
Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School, Boston, MA, USA

Center for Robotic Surgery, Brigham and Women's Health Care, Center for Infertility and Reproductive Surgery, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA
e-mail: agargiulo@partners.org

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_8,

gynecologic subspecialties. As a consequence, at more than 10 years from Professor Azziz's appeal for the need of a strong and vocal reproductive surgery contingent in our health system, the number of reproductive endocrinologists offering the full range of endoscopic reproductive surgery to their patients is probably no higher than in 2002. Indeed, in spite of renewed appeals to promote the specialized role of reproductive surgeons in modern fertility care [2], a culture of disconnected care has somehow seeped into our subspecialty, whereby it is currently acceptable for complex reproductive surgery cases to be referred to minimally invasive general gynecology practices so they can have their procedures done laparoscopically. While the intention may be noble, the action is not always in the best interests of patients. Only a reproductive endocrinology subspecialist can effectively tailor the timing and extent of all medical, surgical, and ART interventions to each patient's unique reproductive endeavor. Take the common example of a 40-year-old nulligravida with borderline ovarian reserve, a new radiologic diagnosis of bilateral endometriomas, hydrosalpinges, and a sizable subserosal myoma. She is likely to receive two very different operations at the hand of a general gynecologist and of a reproductive endocrinologist. While laparoscopic excision of hydrosalpinges, stripping of endometriomas, and myomectomy would be reasonable procedures to consider, they would be the wrong choice in this particular case. Reproductive subspecialists epitomize the minimalist approach to gynecologic surgery: they would favor tubal interruption with biopsy and partial coagulation of the endometrioma and would avoid a myomectomy unless absolutely necessary. The aim of such an operation should not be complete eradication of all detectable pelvic pathology but rather preservation of ovarian reserve and swift triage to ART. When the ultimate goal is to potentiate the conception and birth of a healthy child, a deep knowledge of reproductive endocrinology and infertility is fundamental to effective reproductive surgery. It would therefore be hypocritical to pretend that the status quo of reproductive surgery in our country is adequate and sustainable.

Alas, we are a subspecialty on the verge of relinquishing an essential aspect of our expertise in order to remain true to our values. That is to say that, as a subspecialty, we have long rejected open pelvic surgery and the unacceptable burden of adhesions that it entails [3, 4], yet the majority of us struggle to adopt advanced laparoscopy. Loss of three-dimensional (3D) vision, diminished haptic feedback, counterintuitive motion of the operative instruments, loss of wristed movements, tremor amplification, and unfavorable surgical ergonomics render advanced laparoscopic procedures difficult to master. Reproductive surgery entails extensive and precise suturing (as in myomectomy and tubal reconstructive surgery) and complex anatomical dissection (as in excision of advanced-stage endometriosis). Both tasks tend to be particularly challenging in a conventional laparoscopic environment. An uncompromised laparoscopic approach that replicates open microsurgical technique may be virtually impossible for all but the most skilled and practiced minimally invasive surgeons. The available ethical choices for reproductive specialists until recently were to learn and maintain expert conventional laparoscopic skills or to refer patients to minimally invasive gynecologists. The advent of computer-assisted laparoscopy has ushered in a new and appealing solution to this ethical and professional dilemma.

Advanced reproductive surgeries demand a sophisticated level of medical knowledge, surgical judgment, and technical skill. Computer-assisted laparoscopy, commonly known as robotic surgery, combines the intuitive operative environment of open surgery with the minimal invasiveness of laparoscopy. This technology may therefore enable willing reproductive surgeons to apply their specialized knowledge and microsurgical training toward advanced laparoscopic procedures. Reproductive surgeons were, in fact, the first in gynecology to recognize the benefits of robot-assisted surgery, while early prototypes were still in development, using the now discontinued Zeus platform to demonstrate the feasibility of robot-assisted laparoscopic tubal reanastomosis in 1999 [5]. The United States Food and Drug Administration (FDA) subsequently approved the da Vinci Surgical System (Intuitive Surgical, Sunnyvale, CA) for use in gynecologic surgery in 2005. This system and

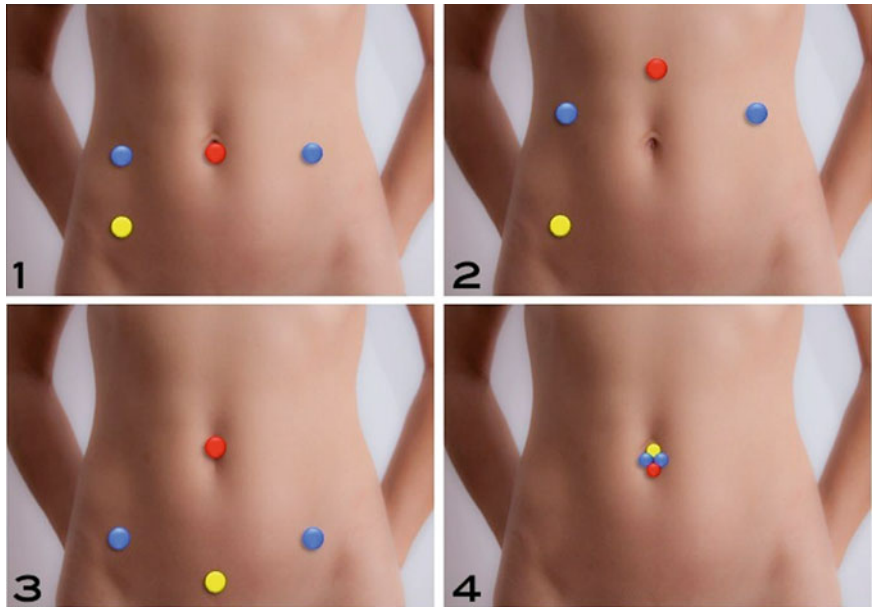

Fig. 8.1 Port placement for robot-assisted reproductive surgery. (1) Standard two-arm configuration with assistant port in the *right lower* quadrant. *Lower* assistant port makes needle exchange safer and allows conventional laparoscopic operation in the "vertical zone" if needed. (2) Standard two-arm configuration for large pathology: this is what we use for our hybrid procedure (see Fig. 8.4); a third robotic arm is placed through a *left* side port in some of these cases. (3) Cosmetic configuration with suprapubic assistant port and low positioning of the bilateral robotic trocars. (4) Umbilical incision for single-port procedures. (Key: *red* = camera port, *yellow* = assistant port, *blue* = robotic ports)

its newer variations (da Vinci S, Si and Si-e) remain the only currently approved robotic surgical platforms for laparoscopic surgery available in the USA, although it is hard to imagine that competing technology will not become available soon.

Current robotic surgical systems consist of three main elements: a single or double remote surgeon's console, a three- or four-arm patient-side robotic cart, and a vision cart. The surgeon employs a computer-aided interface to remotely control specially designed instruments through the passive bedside robot. Following standard abdominal insufflation, a primary cannula is inserted at or above the umbilicus. Subsequently, two or three dedicated 5-mm or 8-mm robotic instrument trocars plus an assistant port are placed as necessary for the planned procedure (Fig. 8.1). The arms of the patient-side robotic cart are connected to the cannulas. The primary surgeon controls an 8.5-mm or 12-mm binocular laparoscope and up to three interchangeable robotic instruments while seated at the remote console. Any of the robotic arms can be reassigned to the secondary console for training purposes or to allow the independent movement of all four arms for more sophisticated techniques. Most robotic instruments feature articulated tips, enabling 7 degrees of freedom in motion: grip, insertion, rotation, and pitch and yaw at both the elbow and the wrist. Floating hand controls at the remote console accommodate the surgeon's thumbs and forefingers. These surgical systems use computer technology to overcome the fulcrum effect: they automatically reverse the pitch and yaw, such that the surgeon's natural hand movements are translated into the precise, scaled movements of the robotic instruments. Pedals for activation of energy instruments are also integrated with the console.

Together, the full impact of these technological innovations is greater than the sum of its parts:

computer-assisted laparoscopy allows inexperienced users to complete complex laparoscopic tasks with less training, greater efficiency, and reduced operator workload compared to conventional laparoscopy [6–9]. In a seminal study by Stefanidis et al., medical students were tested on intracorporeal suturing in porcine Nissen fundoplication models [8]. The subjects were asked to place sutures using conventional laparoscopic instruments in one model and robotic assistance in the other (in random order). Robotic assistance significantly improved intracorporeal suturing performance and operating room safety and significantly shortened the learning curve. In addition, robotic assistance significantly reduced operator workload, as assessed by a validated National Aeronautics and Space Administration Task Load Index (NASA-TLX) questionnaire. This decrease in subjective mental and physical demand could improve physician performance and safety in challenging situations and release mental resources for unfamiliar tasks.

Studies comparing the learning curves for actual procedures using conventional computer-assisted laparoscopy will never be available. That is because such studies, in order to be meaningful, would have to replicate many conditions of the above work by Stefanidis on human subjects for entire operations. This would constitute a bioethical nightmare even in the most permissive of health systems. Several robotic surgery teams have reported their learning curves for specific operations. Not surprisingly, the results vary considerably by procedure and by the surgical experience of the team under study. Lenihan et al. showed that the operative time in robotic benign gynecologic cases (mostly hysterectomies) stabilized after a learning curve of 50 cases [10]. Payne et al. confirmed this finding, showing stabilization of operative time for robotic hysterectomy after 75 cases [11]. In contrast, a subspecialty gynecologic oncology team showed that proficiency for robotic hysterectomy with pelvic–aortic lymphadenectomy was achieved after 20 cases [12], and our own study on a high-volume team of reproductive surgeons could not identify any significant learning curve for robotic myomectomy [13]. Importantly, the prevailing success of these robotic teams and the relatively short learning curves identified suggest that the rapid rise to proficiency afforded by computer-assisted laparoscopy in training tasks may also translate to real operations, where safety and reproducibility are paramount.

Computer-assisted laparoscopy brings two additional features that make it uniquely geared toward operating room safety and surgical education (1) enhanced ergonomy and (2) highly sophisticated simulation with objective evaluation. The field of surgical ergonomics has boomed following the introduction of advanced laparoscopy because the extreme ergonomic challenges it creates pose a threat to the health of both surgeons and patients alike. Surgeons may suffer from occupational injury due to musculoskeletal strain due to the physical maneuvers and unfavorable positioning required for laparoscopic pelvic surgery, whereas patient safety may be compromised by the high level of complexity found in advanced laparoscopic cases and a propensity toward distraction in the form of gaze disruption. These important themes will be explored in detail next.

To understand why the dangerous epidemic of musculoskeletal injury caused by laparoscopic surgery has remained relatively silent until recently, we must place it in the right cultural prospective. Physicians have historically thrived in their *deus ex machina* role, no matter how unfavorable the circumstances. From the carnage of battlefield hospitals throughout history, to the heroic fights against the Black Death, leprosy, and smallpox, to the sacrifices of radiation medicine pioneers, we are the cavalry and we know it. However, it seems this cavalry is not faring too well in laparoscopy.

Park and colleagues polled laparoscopic specialists in North America and reported that 86.7 % of them had symptoms associated with musculoskeletal occupational injury [14]. The main predictor of symptoms was high case volume, whereas age, years in practice, and surgeon's height did not have an impact. A separate study by the same group reported that surgical assistants in laparoscopic surgery were also at risk for musculoskeletal occupational hazard [15].

These recent reports highlight the alarming prevalence of a well-known ergonomic flaw in the musculoskeletal requirements of conventional laparoscopy [16].

Computerized surgical platforms are a promising solution to the ergonomic challenges of laparoscopy because they eliminate the unbalanced posture and the neck and shoulder strain of the remote operators. While long-term benefits conveyed by the improved ergonomics of computer-assisted laparoscopy may be speculative for the time being, the need for a form of laparoscopy that is not crippling to the operator is self-evident. Furthermore, prolongation of surgical careers due to decreased occupational injury could permit more experienced senior surgeons to remain in the lead of their teams, to the advantage of patients and disciples alike. Absence of strain on the operator is also likely to allow a more homogeneous and predictable performance in the course of a long operative day or of a busy operative week.

Aside from the already-mentioned improvement of fundamental operative ergonomics, robotic technology eliminates the problem of gaze disruption. Gaze disruption, looking away from the immediate operating field, is a concept that is alien to classic surgery but implicit in the laparoscopic operating environment, where the visual and motor axes are no longer aligned. Advanced laparoscopy accepts gaze disruptions as a necessity, due to frequent instrument exchange, extracorporeal work, and occasional equipment troubleshooting. Such disruptions are more frequent than most realize: during laparoscopic cholecystectomy, 40 gaze disruptions occur in the main operator for every 15 min of operating time [17]. High-frequency gaze disruptions, a necessity introduced by laparoscopic surgery, constitute an interruption of task performance and can lead to surgical errors. A recent study in open cardiac surgery reported an average of 8.1 surgical flow disruptions per hour (about 20 times less than what was reported for laparoscopic cholecystectomy) and still found that they were associated with surgical errors [18]. Current computer-assisted laparoscopy is performed in a visually immersive environment where expert surgeons can complete an entire procedure without ever taking their eyes out of the visor: gaze disruption in robotic surgery is practically nonexistent.

This last comment cautiously introduces unresolved bioethical issues in laparoscopic surgery that may become relevant to the diffusion of computer-assisted laparoscopy. Although digital simulation for laparoscopic surgery has been available for some time, the level of technological innovation and the amount of research and development that is going into simulation for computer-assisted laparoscopy is understandably much higher, given the computerized nature of the platform. Currently, virtual reality simulators are focused on replicating specific repetitive tasks that prepare the surgeon to achieve optimal economy of motion and safe remote handling of the surgical robotic cart. Because current computer-assisted surgical systems involve simultaneous use of all four limbs, achieving and maintaining the seamless integration of the surgeon's body with the remote console's multiple operating interfaces requires time, much like driving a car. Simulators not only facilitate and optimize this stage of training by eliminating the need for a dry lab or an animal facility (and certainly, live patients) but also provide an incredible variety of skill exercises and a fully objective and detailed technical feedback for the benefit of the trainee and the teacher (Fig. 8.2). Full-procedure virtual reality simulation for computer-assisted laparoscopy is an area of active research and development that is sure to provide useful products in the very near future. However, even current skill-focused simulation for computer-assisted surgical systems has been so remarkably impactful that it is considered by most experts to be essential for the future of robotic surgery training [19, 20]. Thanks to the reality, and promising future, of digital surgical simulation many of us prognosticate the obsolescence of that scary adage that summarizes the tired dogma of surgical education: "*see one*, *do one*, *teach one*." We believe that the new adage "*see one*, *digitally simulate until you can replicate what you saw—only then do one*, *teach one*" is more in keeping with modern surgical ethics and just plain smarter.

Fig. 8.2 Integrated digital simulation for computer-assisted surgical system. Clockwise from *left* to *right*: original surgical console with computer "backpack" installed, frame of actual digital simulation and final score screen with itemized performance commentary. (Photographs courtesy of Intuitive Surgical, Inc.)

In summary, it is critical to realize that computer-assisted laparoscopy is still laparoscopy but with a powerful user-interface that enhances safety and reproducibility. If the main reason for the quasi-demise of reproductive surgery was the impracticality of universally transposing microsurgical quality to the minimally invasive arena, then the entry of robotic technology into our operating rooms should mean a rebirth of our subspecialty surgery. In the next sections of this chapter, we analyze the literature calling for a shift away from laparotomy for virtually all fertility-sparing operations and will highlight the applications of computer-assisted surgical platforms in this critical movement.

8.2 Robot-Assisted Laparoscopic Myomectomy

Uterine fibroids, though not always problematic, are a common finding in women of reproductive age. Women frequently seek treatment for fibroids due to abnormal uterine bleeding, bulk-related symptoms, or poor reproductive outcomes. Indeed, submucous and intramural fibroids have been associated with subfertility, implantation failure, and miscarriage [21]. Fibroids have also been associated with later obstetrical complications such as increased risk of preterm delivery, fetal malpresentation, and labor dystocia [22, 23]. Evidence supporting hysteroscopic resection of submucosal myomas to improve fecundity or ART outcome is limited to small retrospective studies and uncontrolled trials, but the results are compellingly in favor of this treatment [22]. Prospective randomized trials supporting the excision of intramural fibroids for reproductive indications alone, however, are lacking. This amplifies the challenge of determining whether or not myomectomy could benefit a patient's path toward conception and healthy childbirth and makes it all more important to involve a reproductive endocrinologist in such decisions. When surgery is deemed advantageous, reproductive specialists should naturally favor a minimally invasive approach to myomectomy whenever possible.

Fortunately, both traditional laparoscopic myomectomy (LM) and robot-assisted laparoscopic myomectomy (RM) offer a safe and effective minimally invasive option for the treatment of symptomatic uterine fibroids in women who desire future childbearing. Compared to abdominal

myomectomy (AM), LM is associated with lower estimated blood loss and hemoglobin drop, decreased postoperative pain, shorter hospital stay, quicker return to normal activities, and fewer overall complications [24]. Three randomized trials additionally suggest improved fertility following LM compared to AM [25–27]. Despite early concerns regarding the integrity of the myometrial repair, the risk of uterine rupture following LM is quite low—between 0.0 % and 0.25 % [26, 27]—and compares favorably to the rate of rupture following AM, which ranges from 0.0 % to 4 % depending on the series [28–30]. The rate of uterine rupture following RM was similarly reassuring (1.1 %; 95 % CI 0.3, 4.7) in a recent multicenter study involving 127 pregnancies and 92 deliveries in 107 women [31].

Unfortunately, conventional LM is a technically demanding procedure, and despite the many compelling statistics in favor of minimally invasive myomectomy, the procedure remains largely underutilized. A recent survey of Canadian gynecologists reported that only 12.7 % of those performing myomectomy in their practice used LM more than 50 % of the time [32]. While not all myomectomies can be laparoscopic, we predict that robotic myomectomy (RM) will reset the modern standard of care such that most women requiring myomectomy will eventually benefit from a minimally invasive approach to the procedure.

The first series on RM was reported by Advincula and colleagues in 2004 [33]. Since then, multiple studies have demonstrated the safety and efficacy of the procedure. Perioperative outcomes are excellent and mirror those of traditional LM [34]. Case-matched comparisons between patients undergoing AM or RM show that RM is associated with lower mean blood loss, fewer complications, and shorter hospital stay [35–37]. In general, RM takes longer than AM and costs more than LM. An important finding across multiple centers, however, is that reproductive surgeons trained in RM are capable of addressing difficult fibroid cases with a tumor burden that would typically call for laparotomy.

A representative study from the Cleveland Clinic compared perioperative outcomes for 393 abdominal myomectomies, 93 laparoscopic myomectomies, and 89 robotic myomectomies and found no significant differences between LM and RM in terms of blood loss, operative time, or hospital stay despite a significantly larger tumor load in the RM group (223 vs. 97 g, $p<0.001$) [37]. Compared to AM, RM required significantly longer operative time (181 vs. 126 min, $p=0.003$), but hospital stay, blood loss, and hemoglobin drop were all significantly reduced despite a similar tumor load (226 vs. 263 g, $p=0.360$). The authors remarked that robotic assistance allowed many would-be abdominal myomectomies to be performed laparoscopically and concluded that RM might improve utilization of a minimally invasive approach to myomectomy.

Our own experience with RM has been similarly transforming. There is no question that expert laparoscopists can complete complex multiple myomectomies without resorting to laparotomy, but it is noteworthy that reproductive endocrinologists can reproduce such results with the aid of computer-assisted laparoscopy. This was illustrated in our study comparing short-term outcomes from 174 RM and 115 LM performed by separate reproductive endocrinology and minimally invasive gynecology teams [13]. Tumor load was substantial in both groups. The median number of fibroids removed was 2 (range, 1–21) in the LM group compared with 3 (range, 1–16) for RM. Median weight of the fibroid specimens was 201 (range, 1–1,473 g) vs. 159 (range, 8–780 g). Median diameter of the largest fibroid was 7.5 (2.2–16.5) vs. 7.3 (3.1–13.8 cm) in the LM and RM groups, respectively. Perioperative outcomes were excellent for both techniques, but median operative time was significantly longer for RM (191 min vs. 115 min). Barbed suture was used in most LM cases but only in 5 % of RM and may have contributed to the observed difference in operative time. More importantly, this study illustrated that an experienced reproductive endocrinology team could address complex laparoscopic myomectomies with computer assistance and achieve perioperative results comparable to those of an experienced minimally invasive gynecology team. This feat deserves serious consideration despite the increase in operative time.

Since the inception of the gynecologic robotic surgery program at Brigham and Women's Hospital in 2006, our team has performed over 500 robotic myomectomies with no conversions to laparotomy. Most of these are performed as same-day surgery with only a small minority of patients requiring overnight observation or inpatient admission. We strongly believe that our fastidious preoperative evaluation has promoted and upheld our 0 % conversion rate to laparotomy.

High-quality preoperative imaging for fibroid mapping is essential to preoperative and intraoperative planning. The main goals of preoperative imaging are to (1) assess the size and location of all myomata relative to the endometrial cavity, (2) rule out diffuse adenomyosis, and (3) identify lesions suspicious for sarcoma. Pelvic ultrasound is just as useful as magnetic resonance imaging (MRI) in the mapping of smaller uterine masses [38]. However, ultrasound is less useful for larger uteri because this modality often fails to adequately define the relationship between fibroids and the endometrial cavity or other important anatomic landmarks. This information is critical for optimizing uterine incisions during the case and for avoiding unintended entry into the cavity or cornual regions. MRI with and without gadolinium enhancement is therefore preferred in such situations. MRI also has a high specificity for identifying adenomyosis and, together with serum LDH, for predicting the presence of leiomyosarcoma [39, 40]. It is essential to identify both of these conditions preoperatively because neither diffuse adenomyosis nor sarcoma would be amendable to conservative excisional therapy by laparoscopy.

In general, candidates for RM at our program are patients with a largest fibroid dimension under 15 cm and with fewer than 15 total fibroids (Fig. 8.3a, b). RM is not offered to patients with diffuse adenomyosis or with an endometrial cavity obscured by fibroids on MRI or to most women whose uterine fundus extends above the umbilicus on physical exam (this depends on adequate space for trocar placement, uterine mobility, and the perceived ability to debulk the uterus laparoscopically before docking the robot).

Our surgical protocols for RM transpose classic AM technique to the laparoscopic environment. This concept is appealing from a reproductive specialist's perspective where accurate myometrial repair and uncompromising microsurgical technique may conceivably lead to superior reproductive and obstetrical outcomes. While we acknowledge that other centers' RM techniques may vary subtly from ours, we offer here a step-by-step description of our RM technique from the moment the patient is in the operating room. This explanation and the accompanying figures are also intended to serve as a general guide for robotic operating room procedures and port placement strategies, which may be applied other robot-assisted reproductive surgeries in addition to RM.

Once in the operating room, the patient is positioned in dorsal lithotomy with both arms tucked parallel to the body in surgical toboggans. Protective foam padding is secured over the face, arms, and thighs. A pelvic examination is performed under anesthesia for final planning of the best surgical approach and trocar placement. An oral-gastric tube is placed to drain the stomach. After the vagina and abdomen are sterilely prepared, a Foley catheter is inserted to drain the urinary bladder and a uterine manipulator with a channel for chromopertubation is placed.

Port placement configuration is estimated preoperatively but may be finally determined after abdominal insufflation. Initial entry can be gained in the left upper quadrant or umbilicus before determining the final placement for the camera arm, which may be placed several centimeters cephalad to the umbilicus or even just inferior to the xiphoid process for very large uteri. Either a 3- or 4-arm configuration may be used depending on whether a third robotic instrument will be needed for uterine positioning (Fig. 8.1, sec. 1). Dilute vasopressin (20 units vasopressin in 40–60 mL normal saline) is injected into the myometrium overlying the first fibroid targeted for enucleation. The robotic patient-side cart is then "docked" at a 30-degree angle to the left side of the operating table, and all robotic trocars and instruments are correctly positioned and inserted under laparoscopic guidance.

After the vasopressin has taken its effect, we create a transverse incision over the myoma using either robotic harmonic shears or a flexible CO_2

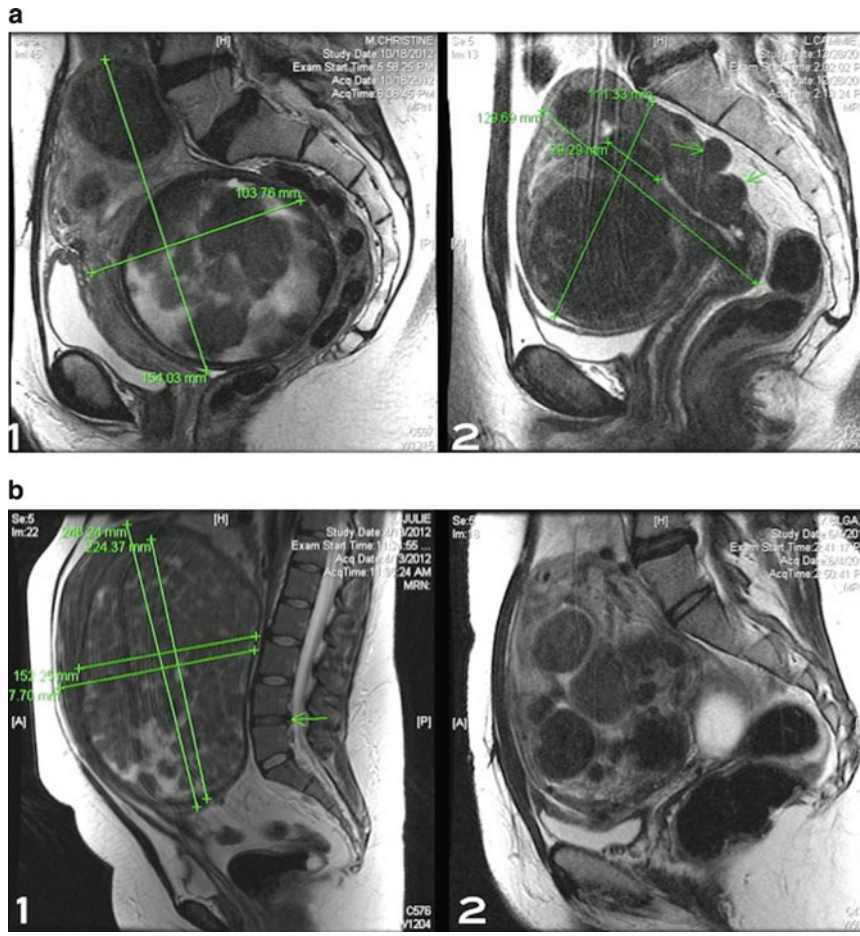

Fig. 8.3 The laparoscopic threshold. (**a**): (1) and (2) show MRI images (T2-weighted sagittal projections) of typical surgical candidates for RM variant at our center. Myomas in the 1–10 cm range are effectively enucleated with this technique in our experience. (**b**): MRI images (T2-weighted sagittal projections) from patients who are not currently considered good candidates for RM at our center. (1) The tumor (22×15 cm) is too large to be safely addressed. (2) The uterus is studded with too many myomata to address laparoscopically ("bag of marbles" appearance)

laser fiber, which have minimal thermal spread, and restrict the use of monopolar robotic shears to postreproductive patients desiring uterine preservation. We prefer transverse uterine incisions when possible because they are more easily repaired than longitudinal incisions and are less likely to transect the arcuate vessels providing blood flow to and from the fibroid and its surrounding myometrium. Robotic tenaculum forceps are used to grasp the fibroid for stabilization, positioning, and traction during the enucleation. A robotic Maryland or other bipolar fenestrated grasper may provide counter-traction if needed, and robotic instruments may be interchanged between arms if this will facilitate dissection around challenging angles.

The bedside assistant has access to the field via a 5 mm or 12 mm port. The smaller port size may be used if the surgeon and assistant feel comfortable passing sutures (and morcellating) through the primary camera port. Enucleated fibroids are placed into the anterior or posterior cul-de-sac or in the paracolic gutter if very large. A running count of the free fibroids is maintained throughout the case. If multiple small fibroids are removed, there we secure them by passing a

suture (polyglactin 910 or polypropylene) on a Keith needle through each of them so that they are not lost in the abdomen before morcellation.

Suturing is performed with a mega or large robotic needle driver in Instrument Arm 1 and a large needle driver in Instrument Arm 2. We strive to remove as many fibroids as possible through each myometrial incision so as to minimize the extent of trauma to the uterus as a whole. Careful preoperative review of the MRI is essential to maximizing the benefit of each incision. Immediate repair of the uterine incisions after fibroid enucleation minimizes blood loss. Chromopertubation may be performed to test for entry into the endometrial cavity. We repair any visible endometrial defect with a running suture of 3-0 poliglecaprone 25 to decrease the risk for intrauterine synechiae or fistula formation. We currently use self-retaining barbed suture, namely Quill (Angiotech, Vancouver, BC) or V-Loc (Covidien, Mansfield, MA), for almost all RM repairs—especially those involving very large myomata. The robotic platform also facilitates intracorporeal knot tying when using conventional suture (polyglactin 910): we still prefer this technique for delicate repairs, such as those of retroperitoneal or cervical myomata. We close the uterine serosa with a baseball stitch using barbed suture or 3-0 poliglecaprone 25. If using a 4-arm configuration, the robotic tenaculum may be used to optimally position or stabilize the uterus during the repair.

The robot is then undocked, and the fibroids are morcellated through either the assistant port site (suprapubic or right lower quadrant) or the umbilical port site. The latter avoids enlargement of assistant port site when a 5-mm trocar is in place, and a standard 5-mm laparoscope can be used through one of the 8-mm robotic ports. We rarely morcellate with the robot docked. After complete hemostasis is assured, Interceed (Ethicon, Somerville, NJ) is placed over the serosal repairs as an adhesion barrier.

Several variations in our RM technique allow for an added degree of individualization toward the patient and pathology at hand. Hybrid (conventional plus robotic) laparoscopic myomectomy consists of conventional laparoscopic enucleation of the largest one or two fibroids followed by swift docking of the patient-side robotic cart for repair of the defect and subsequent enucleation and repair of the smaller fibroids (Fig. 8.4). The hybrid technique works best for myomata >10 cm in diameter, as conventional laparoscopy allows for easier operation in the upper abdomen, which is required for some large myomata (Fig. 8.1, sec. 2). A rigid 10-mm laparoscopic tenaculum also allows the operator to exert more traction than the articulated robotic tenaculum and provides a degree of tactile feedback during the enucleation. Hybrid RM should only be performed by surgical teams that are comfortable with conventional laparoscopic myomectomy techniques and seamless docking of the robot (Fig. 8.1, sec. 2.).

Though our standard port configuration for RM uses small abdominal incisions, many patients find the resulting upper abdominal scars to be less desirable than those which may be more readily concealed below the waist line [41]. We have therefore developed a "cosmetic approach," which allows us to respect this patient preference in women with a smaller tumor load (Fig. 8.5). This technique uses a 3-arm configuration with lower placement of the operating ports and the use of a suprapubic assistant port (5 mm or 12 mm) as discussed above (Fig. 8.1, sec. 3). Incisions for the 8-mm robotic trocars are placed about 3 cm medial and just cephalad of the iliac spines. Of note, the lower and more lateral placement of the robotic arm ports changes the angle of the instruments with respect to the target anatomy and consequently makes the necessary surgical maneuvers more challenging than with conventional (higher) port placement. Use of a 15-cm primary trocar elevates the camera arm an extra 3 cm away from the abdomen and adjacent instrument arms. Assistance from the suprapubic port is facilitated by the use of 25-cm minilaparoscopy instruments to reduce external collisions with the robotic camera arm.

Finally, advanced robotic teams may be able to offer an even more cosmetic approach with robotic single incision laparoscopic robotic myomectomy SIL-RM (Figs. 8.6 and 8.1, sec. 4). We recently reported on two successful cases

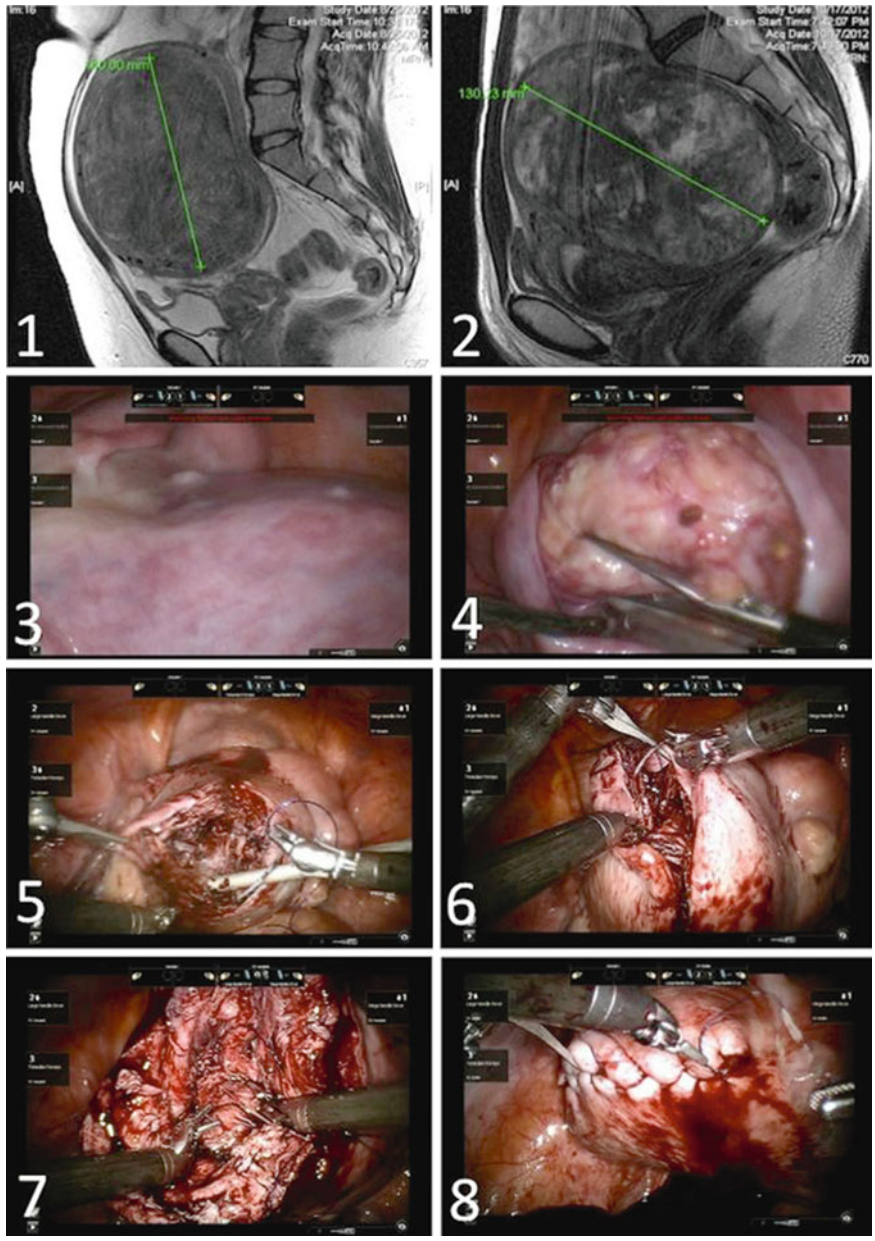

Fig. 8.4 Hybrid robotic myomectomy. (1) and (2) show MRI images (T2-weighted sagittal projections) of typical surgical candidates for the hybrid RM variant at our center. Myomas in the 10–15 cm range are more effectively enucleated with this technique in our experience. (3) Standard laparoscopic camera is used to guide enucleation. (4) The surgeon creates a transverse incision over the myoma using a harmonic scalpel; the 10-mm laparoscopic tenaculum is used to manipulate the fibroid during enucleation. (5) The robot is docked onto the patient and the repair of deep defects is swiftly carried out with barbed suture. (6) This case had two incisions performed during the conventional laparoscopy phase: the smaller one is quickly closed. (7) Attention is brought to the second incision and closure in layer is performed. (8) Suturing the serosal layer with barbed suture is safe provided that an infolding suture line is created (baseball stitch). Smaller myomata are addressed later in the operation

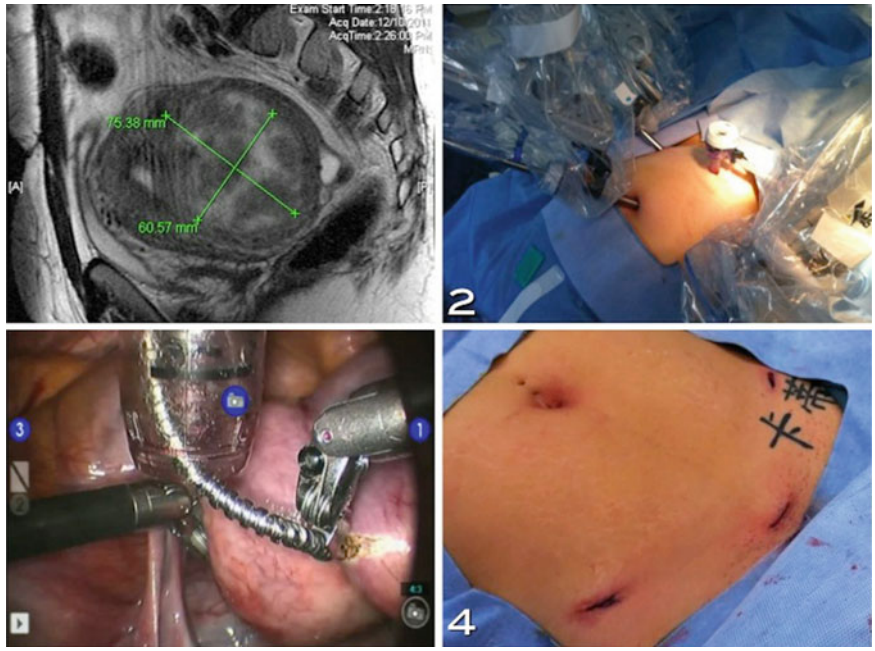

Fig. 8.5 Cosmetic robotic myomectomy. (1) MRI image (T2-weighted sagittal projection) of typical surgical candidates for cosmetic RM variant at our center. Myomas in the 1–8 cm range are effectively enucleated with this technique in our experience. (2) The bedside assistant has access to the operative field via a suprapubic assistant port. (3) The suprapubic assistant port is adequate for assistance, passage of needles and passage of the laser fiber. (4) When the abdomen is desufflated all three lower incisions fall below the level of the anterior-superior iliac spines

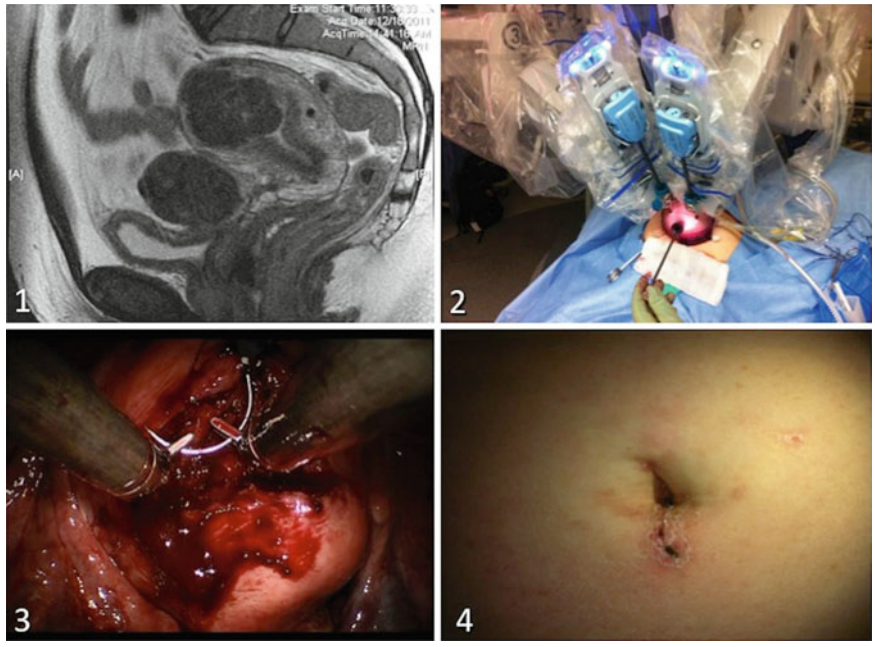

Fig. 8.6 Single incision robotic myomectomy. (1) MRI image (T2-weighted sagittal projection) of typical surgical candidates for single incision RM variant at our center. Myomas in the 1–6 cm range are effectively enucleated with this technique in our experience. (2) The GelPOINT device allows placement of four laparoscopic channels, including an assistant port (most cephalad). (3) The coaxial technique is made possible by the wide yaw of the 8 mm robotic instruments. (4) At 2 weeks from surgery results are already cosmetically remarkable. The best cosmetic results are certainly obtained in women with a significant abdominal pannus and deep umbilicus

utilizing this ultra-minimally invasive technique [42]. Apart from reducing the number of visible incisions, clinical advantages of robotic SIL-RM may include decreased postoperative pain and reduced risk of herniation and superficial vessel and nerve injury. Successful application of the SIL-RM technique involves (1) use of Instrument Arms 1 and 3 with Arm 2 folded around the main column of the patient-side cart and (2) a periscopic "up" approach with a 30-degree robotic laparoscope to allow room for a 5-mm assistant port in the GelPOINT Advanced Access Platform (Applied Medical, Rancho Santa Margarita, CA).

Regardless of the type of RM performed, we prefer to see patients for a postoperative visit approximately 2 weeks after surgery to ensure that their recovery has been uneventful. Patients may resume intercourse as early as 2 weeks postoperatively but are specifically counseled to use contraception for a minimum of 3 months to allow the myometrium time to heal prior to pregnancy. We recommend that patients with a large intramural myomectomy or with a myomectomy that has reached the endometrium (even without entering it) undergo a cesarean delivery. In patients of reproductive age whose endometrial cavity was entered during RM, we recommend a follow-up outpatient hysteroscopy to rule out intrauterine synechiae.

In conclusion, while the choice of AM raises ethical questions when LM is feasible, a technically uncompromised LM that is the exact replica of an open microsurgical myomectomy is arguably one of the most technically demanding pelvic operations ever conceived and is likely to remain out of the practical reach of most subspecialists. Several studies now indicate that RM is as safe and effective as conventional LM. Ultimately, every laparoscopic surgeon—advanced or basic—has a personal threshold for open surgery. With proper case selection, however, RM may be able to replace AM in most instances and should offer an appealing alternative to LM for most reproductive surgeons. As reviewed here, RM generally requires additional operating room time compared to AM and additional resources compared to conventional LM. However, we find it likely that many reproductive endocrinologists would accept these as reasonable investments toward a quality surgical procedure that raises their laparotomy threshold, adheres to classic microsurgical principles, and facilitates seamless subspecialty-level reproductive care for their patients.

8.3 Tubal Reanastomosis

One in five women under the age of 30 at the time of tubal sterilization later regrets her decision [43], Even so, tubal reanastomosis in the age of assisted reproduction appears to be going the way of the dodo [44]. This is most unfortunate. Reproductive endocrinology and infertility practices should be able to offer this technique as an option for women with no other apparent cause of infertility and for whom multiple gestations are not acceptable or assisted reproduction is otherwise not desired, ethically acceptable or attainable. In a cost-conscious environment where neonatal intensive care costs related to multiple premature deliveries vex our health system, our conscience—or third-party payers—should prompt more of us to offer surgical sterilization reversal over in vitro fertilization when appropriate. In order to compete with assisted reproduction, this operation should be minimally invasive, effective, and competitively priced.

Tubal reanastomosis generally aims to reestablish the patency of a 1–2 mm lumen. Classic microsurgical techniques employ an operative microscope and ultrafine sutures to produce an anatomically correct, tension-free anastomosis. A select group of reproductive surgeons have been able to replicate this microsurgical technique laparoscopically and have reported clinical results comparable to those of open microsurgery [45]. Still, most reproductive surgeons would agree that the technical challenges posed by laparoscopic tubal reanastomosis are formidable. The rate of conversion to laparotomy was 5 % even at one high-volume center [46]. Surgical case volume is an issue while developing and maintaining one's laparoscopic skill set for tubal reanastomosis—and perhaps even more so when teaching this relatively rare procedure.

Table 8.1 Pregnancy and delivery rates within 2 years of RTR

Patient age (and number)	Number and % with at least one pregnancy (95 % CI)	Number and % with at least one delivery (95 % CI)
≤35 ($n=33$)	$n=30$; 91 % (76–98 %)	$n=29$; 87.9 % (72–97 %)
36–39 ($n=32$)	$n=24$; 75 % (57–89 %)	$n=21$; 65.6 % (47–81 %)
40–42 ($n=16$)	$n=8$; 50 % (25–75 %)	$n=7$; 43.8 % (20–70 %)
≥43 ($n=12$)	$n=4$; 33 % (10–65 %)	$n=1$; 8.3 % (<1–38 %)
Total ($n=93$)	$n=66$; 71 % (61–80 %)	$n=58$; 62.4 % (52–72 %)

Age stratification according to the Belgian Register for Assisted Procreation
Note: 4/97 patients were lost did not complete the 24 month follow-up and were not included in the final analysis
Redrawn from Caillet et al. Fertil Steril. 2010, with permission

Computer assistance could help to improve the practicality and diffusion of this valuable laparoscopic technique.

Several teams have published on the safety, feasibility, and effectiveness of robotic tubal reanastomosis (RTR). Surgeons at the Cleveland Clinic first described the procedure on the now discontinued Zeus robotic system [47] and later compared RTR with the Da Vinci robotic system ($n=26$) to microsurgical reanastomosis via outpatient minilaparotomy ($n=41$) [5]. Pregnancy rates (61 % robotic vs. 79 % minilaparotomy), ectopic rates, and hospitalization times were not significantly different, but operative times were longer and direct procedure costs were higher in the RTR group. Return to work however was shorter by 1 week in the RTR group. Dharia-Patel et al. performed a similar prospective cohort study (RTR vs. open reanastomosis) with comparable results [48].

Although most surgeons counsel patients based on data from their practice, we find that the best published data to counsel women regarding their age-dependent chance for success following RTR is from Caillet et al. [49]. This large retrospective cohort study analyzed pregnancy outcomes for 97 women aged 24–47 years (median age 37 years) who underwent RTR. It should be noted that all women had normal follicular phase FSH levels and normal male partners' semen analyses. The overall pregnancy and live birth rates at 2 years after surgery were 71 % and 62 %, respectively. Nearly 88 % of women <35 years old and 44 % of women aged 40–42 years delivered at least one child during the follow-up period (Table 8.1).

We perform RTLR by a modified version of the procedure described by Degueldre et al. [50]. The basic steps of this technique, illustrated in Fig. 8.7, have been published elsewhere [51]. Briefly, a uterine positioning system with chromopertubation capability is placed. Laparoscopic port placement follows the same scheme illustrated above for our cosmetic RM. Placement of a third robotic arm is not possible in this configuration: when more complex anatomy or a less experienced team are involved, a more conventional robotic port placement is recommended. This way Prograsp robotic forceps can be operated through the third instrument arm for improved exposure and tissue stabilization. Side docking of the robotic patient-side cart allows easier access to the suprapubic assistant port and the uterine positioning system. Robotic instruments include Potts Scissors and MicroBipolar forceps during the initial step of tubal stump preparation and two Black Diamond Micro Forceps during suturing. Ultrafine (1:5) downscaling is recommended for da Vinci S and fine downscaling (1:3) for da Vinci Si. Dilute vasopressin is injected into the proximal and distal segments of the mesosalpinx to allow optimal hemostasis. Following mobilization and partial amputation of the tubal stumps, patency of the proximal stump is confirmed by chromopertubation. We employ a graduated 3–5 French endoscopic retrograde cholangiopancreatography (ERCP) cannula as a tubal stent to provide anatomic orientation and to help identify the tubal lumen during suture placement. The mesosalpinx is approximated with 1–2 sutures of 6-0 Vicryl in order to take the tension off the reanastomosis line.

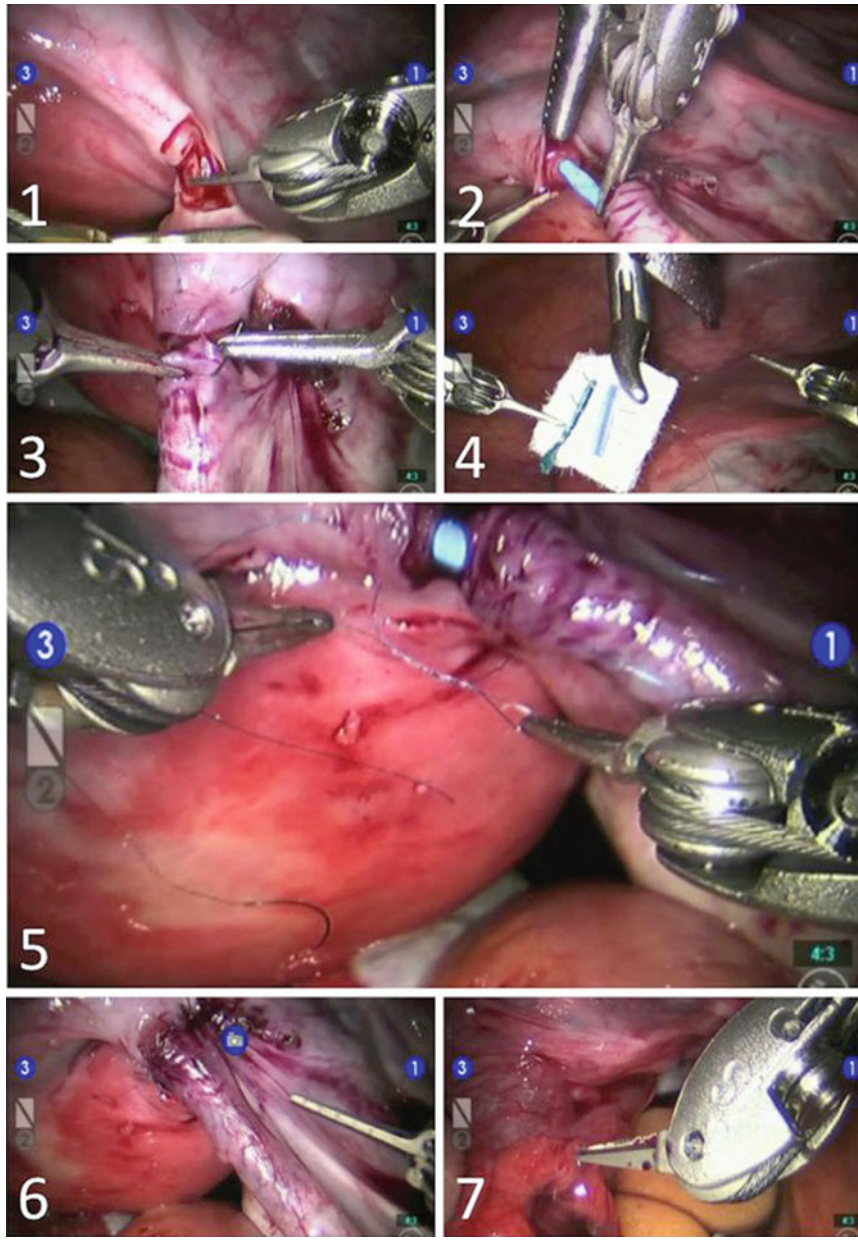

Fig. 8.7 Robotic tubal reanastomosis (RTR). (1) The proximal and distal tubal segments are identified and mobilized with microbipolar forceps and Potts scissors. (2) The tubal segments are cannulated and stabilized with a graduated ERCP catheter. (3) The tubal segments are reanastomosed with interrupted sutures of 8-0 Prolene using Black Diamond forceps. (4) Needles are passed on surgical patties under complete visualization to avoid needle loss. (5) Sutures are serially tied over the guide catheter, resulting in an anatomically correct, tension-free anastomosis as shown in (6). (7) Tubal patency is demonstrated by chromopertubation

The tubal stumps are then approximated next with four interrupted 8-0 Prolene sutures placed at 3, 6, 9, and 12 o'clock in the muscularis and mucosa, with great care to place the knots on the outside of the lumen. The serosa is reapproximated with interrupted 8-0 Prolene sutures if needed. Chromopertubation must confirm tubal patency at the completion of the procedure.

In conclusion, laparoscopic tubal reanastomosis, with or without computer assistance, is a highly specialized surgery. The ASRM advises that it should only be attempted by those who are very facile with laparoscopic suturing and have extensive training in conventional tubal microsurgery [52]. Several authors have demonstrated that RTR is safe, effective, and reproducible. Our unpublished experience with conventional and RTR techniques over the past 15 years indicates that robotic surgery greatly facilitates the learning and successful completion of this challenging operation on the part of our trainees. Well-designed studies addressing this specific question may never become available, due to the fact that advanced laparoscopic surgeons with access to robotic technology are unlikely to continue training future generations of surgeons on conventional laparoscopic reanastomosis. Moreover, the popularity of long-acting reversible contraception, hysteroscopic sterilization, and assisted reproduction will likely diminish the opportunities for individual surgeons to acquire and maintain expertise in tubal reanastomosis. Therefore, the use of computer-assisted technology could gain even greater importance for the future of this minimally invasive procedure.

8.4 Surgical Management of Endometriosis

Nowhere is the direct involvement of reproductive endocrinologists in the operating room likely to be more impactful as in the management of severe pelvic endometriosis in women who desire future childbearing. Indeed, this is one of the most controversial areas of reproductive surgery. While laparoscopic destruction of minimal to mild endometriosis may improve fecundity [53–55], benefits are less clear for advanced stage endometriosis [56–61]. Reproductive endocrinologists must carefully weigh the benefits of every surgical act against the risks of iatrogenic harm in the context of the patient's specific symptoms and her immediate and future reproductive plans.

By far the most complex aspect of the surgical management of this condition is the special case of cystic ovarian endometriosis (endometrioma). Depending on the presentation, fertility status, and age of the patient, excision of an endometrioma can be mandated or contraindicated. A stubbornly radical approach to recurrent endometriomas in a nulligravida in advanced maternal age may only temporarily improve the symptoms of this chronic inflammatory condition, but at the same time condemn her to procreate with donor oocytes. On the other hand, the assumption that a persistent 5-cm complex cyst in a young IVF patient is a benign endometrioma may cause delayed diagnosis and dissemination of an ovarian malignancy. For these reasons, reproductive endocrinologists must be able to deliver expert first-line surgical treatment for endometriomata in women of reproductive age, from indication to execution.

Ovarian cystectomy involves "stripping" of the cyst wall from the normal ovarian tissue while causing minimal trauma. Dissection of the cyst wall away from normal ovarian cortex can be technically challenging at laparoscopy, particularly for endometriomas. These cysts lack a true cyst wall but have instead a pseudocapsule derived from stretching and inflammation of a portion of ovarian cortex. As such, stripping of an endometrioma always causes a loss of primordial follicles. Moreover, the deepest portion of the pseudocyst is often found in close association with the vascular hilum of the ovary. This can contribute to more serious functional ovarian loss if thermal energy is employed to achieve hemostasis [57, 58, 60].

There are no published studies specifically describing the use of computer-assisted surgery in the management of endometriomas. In our experience, computer assistance facilitates ovarian cystectomy by offering 3D visualization of tissue planes and by facilitating the precise application of traction vectors during stripping procedures. We preferentially use cold sheers or CO_2 laser to incise the ovarian cortex overlying the tumor when necessary [62]. Robotic excision of endometriomas is performed by careful use of opposing forceps. In cases where preservation of ovarian reserve is essential, we employ a recently described technique of partial stripping (removing approximately 85 % of the pseudocapsule

area) followed by ablation of the deepest area of the endometrioma, overlying the hilar vessels. Donnez and colleagues found that this hybrid excision–ablation technique was not associated with a postoperative decrease in antral follicle count and rarely resulted in the finding of normal ovarian follicles at histologic evaluation of the excised cyst wall (2 %) [63]. Similar results were reported by Muzii and Benedetti Panici in their version of this technique employing electrocautery [64]. This group likewise modified their technique for surgical management of endometriomas after finding that normal primordial ovarian follicles are most concentrated at the base of the endometriotic pseudocyst, overlying the ovarian hilum [65]. They also histologically mapped the inner wall of the endometrioma and found that it is covered by endometriotic tissue on 60 % of the surface, with a mean value of maximal depth of endometriosis penetration of only 0.6 mm [66]. Given the distribution of normal and pathologic ovarian tissue and the reassuring reports on postoperative ovarian reserve described above, we agree that a conservative excision–ablation approach to benign ovarian cystectomy is conceptually ideal.

Our version of this technique—adapted for the robotic system—involves the use of a flexible hollow fiber CO_2 laser device (Fig. 8.8). The flexible fiber allows full use of the 7 degrees of freedom of the robotic system, which results in highly precise and very safe use of this energy form [62]. An 8-mm assistant port delivers the flexible fiber (contained within an armored introducer) into the abdominal cavity and still allows use of 5-mm assistant instruments through the same port. A robotic needle driver is locked into the tip of the fiber introducer. When necessary, photonic energy or cold shears are used to create a primary incision in ovarian cortex until the endometrioma is exposed. Precise plane dissection between the pseudocyst wall and the ovarian stroma is mostly achieved by blunt technique providing appropriate traction. Occasional areas of adhesion are lysed by utilizing low energy setting of the laser. Following excision, the ovarian bed is irrigated and hemostasis is established. Small bleeders can be coagulated with the help of the divergent laser beam, which provides a superficial coagulative effect when held at a distance from the target. If necessary, localized figure of eight sutures of 6.0 Vicryl or similar absorbable suture are used to control focal bleeding. Hemostatic matrix or other local hemostatic agents should also be considered as a worthwhile alternative to electrocoagulation in cases of more persistent bleeding. In general, no other form of energy is needed to secure hemostasis, thus aiding in ovarian tissue preservation. The ovarian defect can be gently approximated with 3-0 Vicryl in a continuous, unlocked, baseball-stitch. We wrap the completely hemostatic ovary in an adhesion barrier at the end of this procedure.

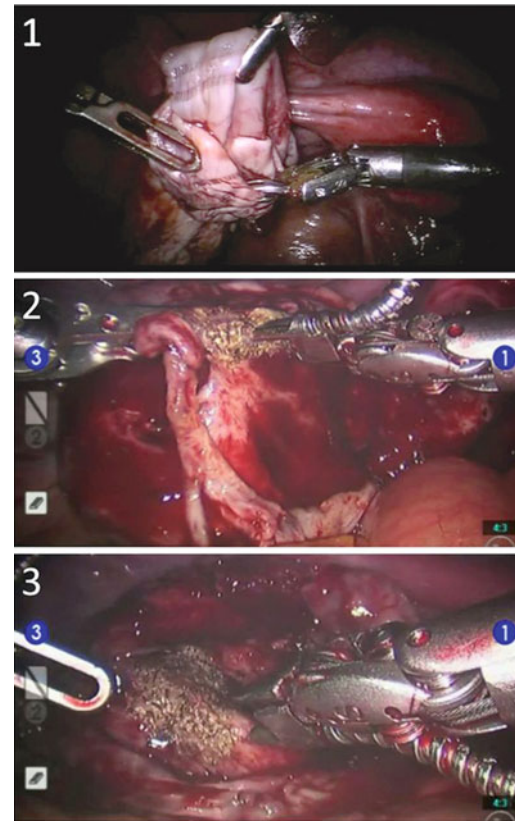

Fig. 8.8 Robotic excision of endometrioma with partial stripping and coagulation of the base. (1) A cosmetic port setup as the one described in Fig. 8.1, sec. 3 is adequate for this operation: the assistant can help during the stripping by immobilizing the pseudocyst. (2) A flexible CO_2 laser fiber is used to precisely excise the large portion of the endometrioma cleaved off of the ovarian stroma. (3) The base of the endometrioma, overlying the ovarian hilum, is left in place: its internal surface is ablated with CO_2 laser

In summary, standard laparoscopic techniques for ovarian cystectomy carry inherent reproductive risks and this is particularly true for endometrioma, where surgical technique and level of execution can make a big difference [67]. Thus, a search for alternative methods to effectively remove these ovarian cysts while minimizing risks of recurrence and ovarian failure seems reasonable. Research on clinical outcomes following computer-assisted conservative adnexal surgery is needed.

Reproductive surgeons are well aware that endometriomas are often found in the context of other features of advanced-stage endometriosis, such as dense adhesions distorting peritoneal and even retroperitoneal anatomy, as well as deep infiltrating endometriosis. Several case series describe the successful application of robotic surgery to severe endometriotic disease involving the bladder, bowel, and ureters [68, 69]. A retrospective study published by a team of high-volume minimally invasive surgery experts compared robotic and conventional laparoscopic treatment of endometriosis [70]. Both methods were equally safe. Although operative times were longer in the robotic group (191 vs. 159 min), the authors were positively impressed with the overall value of robotic assistance in managing advanced-stage endometriosis: they reported no conversions to laparotomy in nine cases of stage III–IV endometriosis. Computer-assisted surgery may enable more reproductive endocrinologists to offer advanced endoscopic procedures to their patients in instances when the alternatives might have been laparotomy or referral to a nonreproductive specialist. The caveat with severe endometriosis is that no degree of computer assistance can ever substitute for strong fundamentals of surgical anatomy, technique, and judgment.

8.5 Surgical Fertility Preservation: Ovarian Transposition, Ovarian Tissue Cryopreservation, and Transplantation

It is now well documented that treatment for malignancy and certain benign medical conditions may threaten a woman's fertility. Reproductive tract tumors may require removal of the uterus with or without the ovaries, while many other conditions may require gonadotoxic radiation or chemotherapy, which can damage a woman's ovaries or uterus and place her at risk for infertility and premature ovarian failure. Reproductive endocrinologists have a critical role to play in the pretreatment counseling of women facing gonadotoxic therapies and should work diligently with oncologists and other medical providers to ensure that these patients are fully aware of their options for fertility preservation. Some—but not all—women will be candidates for oocytes or embryo cryopreservation. When feasible and successful, these techniques provide a reasonable level of hope for patients to produce genetic offspring following gonadotoxic therapies. The advantages of these techniques are that they take advantage of established assisted reproductive technologies. The disadvantages are the time required for controlled ovarian hyperstimulation and oocyte retrieval (and possible delay in cancer treatment), the direct cost to the patient, the possibility that no pregnancy is achieved with the finite number of gametes or embryos cryopreserved, the fact that ovarian steroidogenic function is not preserved, and the exclusion of prepubertal girls. Surgical fertility preservation techniques, both proven and experimental, may address some of these problems. In this section, we address fertility-sparing surgery from the perspective of a reproductive surgeon. We believe that providers should be aware of these options, their indications, and limitations. This brief overview of surgical fertility preservation illustrates the feasibility of laparoscopic techniques and highlights the potential application of computer-assisted laparoscopy in fertility-sparing surgery.

Women who will receive pelvic radiation for malignancy, such as lymphoma, cervical, anal, rectal, and urinary tract cancers, may benefit from ovarian transposition. Ovarian transposition aims to spare the ovaries from sterilizing doses of radiation by suspending them away from the radiation field. The utero-ovarian ligaments are transected, allowing the ovaries to be moved out of the pelvis and fixed to the abdominal wall peritoneum [71–73]. Either a high lateral suspension to the paracolic gutters or an anteromedial

suspension (3–4 cm above the umbilical line) may be performed, depending on the planned radiation field [71, 74]. When necessary, the proximal fallopian tube may also be transected to facilitate a high transposition. However, many surgeons prefer to leave the fallopian tubes intact whenever possible because spontaneous pregnancies may still be achieved [51, 71]. If patients should later need ART, transabdominal oocyte retrieval is an effective option for patients whose ovaries can no longer be accessed transvaginally [75].

Ovarian transposition is a relatively straightforward procedure, which can be performed by laparotomy or by laparoscopy. However, the laparoscopic approach conveys obvious advantages in terms of immediate recovery and healing. Standard laparoscopic oophoropexy requires endoscopic suturing or the use of endoscopic tacks to fix the ovary to the peritoneum. A robot-assisted approach may be used to facilitate the laparoscopic suturing needed for this procedure. Molpus and colleagues were the first to describe a case of robotic laparoscopic ovarian transposition in a 32-year-old woman requiring pelvic radiation for cervical cancer after radical hysterectomy and pelvic lymphadenectomy [76]. A key aspect to their approach was that they introduced the robotic laparoscope through a suprapubic trocar in order to facilitate visualization of the adnexa and upper pelvis. Their patient had significant pelvic adhesions following her prior surgery. Computer-assisted laparoscopic adhesiolysis, retroperitoneal dissection, and ureteral dissection allowed them to safely achieve a high lateral transposition in which the ovaries were suspended to the ipsilateral paracolic gutters without dividing the fallopian tubes. This case and others like it demonstrate that robotic ovarian transposition is feasible and would be an obvious choice when the robot is already in use for concurrent procedures and a high resuspension of the ovaries is required [76, 77]. As in the case presented by Molpus, robot-assistance may also prove advantageous when the need for retroperitoneal dissection or extensive adhesiolysis is encountered.

Computer-assisted laparoscopy may also find use in the key steps of ovarian tissue harvesting and transplantation for women undergoing ovarian tissue cryopreservation. This experimental fertility-sparing technique involves the harvesting of whole ovaries, ovarian wedges, or strips of ovarian cortex for cryopreservation and eventual reimplantation and/or in vitro maturation and fertilization of oocytes [78]. Currently, ovarian tissue harvesting is one of the only fertility preservation methods available to prepubertal girls. It may also be useful for women who cannot undergo controlled ovarian hyperstimulation to bank oocytes or embryos prior to gonadotoxic chemotherapy and, unlike these ART options, adds the possibility to restore native ovarian endocrine function, as well as fertility.

Except in instances where laparotomy is necessary for other indications, ovarian tissue harvesting and reimplantation should be performed laparoscopically. The feasibility of laparoscopic ovarian tissue harvesting was well demonstrated by Oktay and colleagues, who harvested ovarian tissue from 52 women without conversion to laparotomy [79]. Mayerhofer and colleagues similarly retrieved ovarian tissue laparoscopically from 81 out of 85 women with no adverse events and concluded that laparoscopy should be the gold standard for the procedure [80].

When patients have been cleared by their oncologists to resume attempts at reproduction, strips of ovarian tissue can be thawed and reimplanted orthotopically (back to the ovary or ovarian fossa) or heterotopically (e.g., under the skin of the arm or the abdomen) [79]. For orthotopic transplantation, most prepare a transplant site on the recipient ovary by removing some of the existing ovarian cortex or by creating a tunnel beneath the cortex so that the tissue grafts can be sutured to decorticated ovarian medulla [79]. Alternatively, strips of preserved ovarian tissue can be sutured to a pocket created in the nearby peritoneum. Graft-site preparation and orthotopic transplantation can be accomplished by an advanced laparoscopic team, but most have resorted to an open approach [81, 82]. Additionally, those reporting laparoscopic variations of the procedure used oxygenated cellulose products as scaffolds to help secure the strips of tissue to the recipient ovary or peritoneum. It remains to be seen whether open or laparoscopic techniques result in better graft survival and function.

However, robotic assistance might resolve the potential difficulties of laparoscopic ovarian tissue transplantation without the need for technical compromises. One case study demonstrated that open techniques for orthotopic ovarian tissue transplantation could be faithfully replicated with robotic assistance [83]. In this example, the robotic surgical team successfully transplanted strips of thawed ovarian tissue on ovarian and peritoneal sites in a 38-year-old woman with ovarian failure following treatment for non-Hodgkin's lymphoma. The patient subsequently experienced return of ovarian function and relief of hot flashes within 6 months.

Ovarian graft function and longevity are generally temporary and appear to depend on ischemic injury and neovascularization. Whole ovary cryopreservation and transplantation have been proposed as a means to circumvent these issues. The concept of ovarian transplantation has been tested and proven in animal models and in a fresh human ovarian transplantation between monozygotic twin sisters [84–87]. In this method, an entire ovary is transplanted to an orthotopic or heterotopic recipient site by microsurgical vascular anastomosis. While no cases of laparoscopic or computer-assisted ovarian transplantation have yet been reported, robotic surgery has already been successfully applied to other procedures requiring vascular anastomoses, including renal transplantation and coronary artery bypass grafting [88, 89]. We thus foresee the possibility of applying this technology to ovarian transplantation in the future.

8.6 Conclusions

Reproductive endocrinology and infertility subspecialists must remain fully engaged in the management of patients seeking fertility preservation and enhancement of natural fecundity or ART success. Such engagement is incomplete when these highly trained providers relinquish their role as reproductive surgeons.

There is no doubt that assisted reproductive technologies and advanced laparoscopic techniques have transformed the role of surgery in the practice of reproductive medicine. In particular, the development of advanced laparoscopy has set high technical standards in fertility-sparing and fertility-enhancing surgery. Laparotomy no longer has a role in reproductive surgery, with rare exceptions.

In fairness to all of us, we should recognize that a technological tsunami has overwhelmed our field in the course of the past generation, broadening our armamentarium to previously unimaginable levels. Reproductive specialists must remain focused to achieve excellence in their high-specialty field: there is no question that a great number of us have chosen to focus on assisted reproduction rather than surgery. Our review explains the possible reasons for the silent retreat of reproductive endocrinologists from the field of reproductive surgery and particularly from the discipline of laparoscopy, which had been synonymous with our field for good part of its early years [90]. We have covered in detail the many levels of ergonomic challenge implicit in laparoscopic surgery: from mechanical limitations due to the fulcrum effect, to musculoskeletal occupational injury, to safety concerns related to gaze disruption in the operating room. We have also highlighted how the retreat of many REI subspecialists from the front line of advanced laparoscopy has created a culture of disconnected care for infertility patients with surgically treatable conditions.

Computer-assisted laparoscopy, a readily available and accessible reality in most medical centers in the USA, represents a practical solution for reproductive endocrinology and infertility specialists to reclaim reproductive surgery as a high-specialty field. As demonstrated in the technical sections of this review, safety and noninferiority studies of robotic surgery compared to conventional laparoscopic surgery are available for every type of reproductive surgery. The AAGL recently proposed that computer-assisted laparoscopy and conventional laparoscopy are to be seen as clinically equivalent [91]. At the same time, the association raises the question of price versus value when considering the higher direct costs of robotic technology compared to conventional laparoscopy. Some caveats come to mind,

however, when interpreting the current evidence related to costs introduced by a widespread utilization of computer-assisted surgery.

First, computer-assisted surgery is a practical ergonomical laparoscopic alternative to open surgery. Because of this, a meaningful cost analysis must consider all societal costs related to open surgery. These include cost related to complications of open surgery and cost of lost productivity of individuals removed from the workforce for extended periods of time. Elimination, or quasi-elimination, of open gynecologic surgery is something that conventional laparoscopy has failed to achieve in the course of an entire generation. Computer-assisted surgery has a realistic potential to change the demographics of surgery on a large scale and to therefore induce substantial savings for society. To date, a study of this scope has not been produced.

Second, the economics of robotic technology are expected to follow trends established by high-end electronic products in the same class—that is to say, a rapid upgrade of technology and an increased affordability over time. The recent AAGL Statement is based on evidence that has accumulated over the first 7 years of gynecologic surgery experience with one specific device whose concept and overall mechanics are over 10 years old. In contrast, those of us with a special interest in the field of surgical robotics can easily predict a different scenario, possibly just around the corner, in which diverse and more advanced robotic products will be competing in an exponentially receptive market. After all, we are merely witnessing the dawn of surgical robotics and computer-assisted laparoscopy. Current detractors of this technology appear to be shortsighted and risk to be eventually marginalized in their surgical profession by failing to recognize the start of a new surgical age. Reproductive endocrinology and infertility specialists in particular should be eager to take advantage of computer assistance to reclaim their place in the surgical arena as the champions of microsurgery, reconstructive surgery, and conservative surgery for all appropriate indications in women of reproductive age.

As highlighted in this review, reproductive surgeons are minimalists: they exercise restraint in the operating room, strong in their deep knowledge of reproductive pathophysiology and in their extensive pharmacological and technological armamentarium. As such, they are likely to remain relatively low-volume, high-specialty operators. They need to provide focused, high-precision, minimally invasive surgical care to the select patients that truly need it. Computer-assisted surgery has the potential to shorten or eliminate learning curves for advanced laparoscopic operations and can maintain surgical skills through easily accessible integrated digital simulation. Therefore, its introduction in a relatively low-volume surgical practice makes even more sense. In terms of its potential to further advance the field of minimally invasive surgery, this rapidly developing technology will soon allow surgeons to bring multiple operative instruments in a patient's abdomen through a single incision that fits within the average umbilicus without the hyperbolic ergonomic challenges of standard single-site laparoscopy. Finally, a combination of computer-assisted laparoscopy and natural orifice transluminal endoscopy promises to provide an ultra-minimally invasive avenue to certain reproductive surgeries.

If REI subspecialists were awaiting a technological quantum leap to empower their leadership in high-specialty reproductive surgery, the time has now come. The obituary of open surgery for benign gynecology has been written, and computer-assisted laparoscopy meets our stringent surgical principles while overcoming the limitations of conventional laparoscopy. In spite, yet because, of the success of ART, the need for highly specialized operators has never been greater. It is only up to us to decide if, as reproductive endocrinologists, we want to be surgeons to our patients. As we say here in Boston: "Fish, or cut bait!"

References

1. Azziz R. Role of reproductive surgeons and the society of reproductive surgeons. Fertil Steril. 2002;78(5):916–7.
2. Adamson GD. The modern role of reproductive surgery. Clin Obstet Gynecol. 2011;54(4):710–9.

3. Practice Committee of American Society for Reproductive Medicine in collaboration with Society of Reproductive Surgeons et al. Pathogenesis, consequences, and control of peritoneal adhesions in gynecologic surgery. Fertil Steril. 2008;90(5 Suppl):S144–9.
4. Gutt CN, Oniu T, Schemmer P, et al. Fewer adhesions induced by laparoscopic surgery? Surg Endosc. 2004;18(6):898–906.
5. Rodgers AK, Goldberg JM, Goldberg JM, Hammel JP, Falcone T, et al. Tubal anastomosis by robotic compared with outpatient minilaparotomy. Obstet Gynecol. 2007;109(6):1375–80.
6. Chandra V, Nehra D, Parent R, et al. A comparison of laparoscopic and robotic assisted suturing performance by experts and novices. Surgery. 2010;147(6): 830–9.
7. Passerotti CC, Passerotti AM, Dall'Oglio MF, Leite KR, et al. Comparing the quality of the suture anastomosis and the learning curves associated with performing open, freehand, and robotic-assisted laparoscopic pyeloplasty in a swine animal model. J Am Coll Surg. 2009;208:576–86.
8. Stefanidis D, Wang F, Korndorffer Jr JR, et al. Robotic assistance improves intracorporeal suturing performance and safety in the operating room while decreasing operator workload. Surg Endosc. 2010; 24(2):377–82.
9. Yohannes P, Rotariu P, Pinto P, et al. Comparison of robotic versus laparoscopic skills: is there a difference in the learning curve? Urology. 2002;60(1):39–45; discussion
10. Lenihan Jr JP, Kovanda C, Seshadri-Kreaden U. What is the learning curve for robotic assisted gynecologic surgery? J Minim Invasive Gynecol. 2008;15(5): 589–94.
11. Payne TN, Dauterive FR. A comparison of total laparoscopic hysterectomy to robotically assisted hysterectomy: surgical outcomes in a community practice. J Minim Invasive Gynecol. 2008;15(3):286–91.
12. Seamon LG, Fowler JM, Richardson DL, et al. A detailed analysis of the learning curve: robotic hysterectomy and pelvic-aortic lymphadenectomy for endometrial cancer. Gynecol Oncol. 2009;114(2): 162–7.
13. Gargiulo AR, Srouji SS, Missmer SA, et al. Robot-assisted laparoscopic myomectomy compared with standard laparoscopic myomectomy. Obstet Gynecol. 2012;120(2 Pt 1):284–91.
14. Park A, Lee G, Seagull FJ, et al. Patients benefit while surgeons suffer: an impending epidemic. J Am Coll Surg. 2010;210(3):306–13.
15. Lee G, Lee T, Dexter D, et al. Ergonomic risk associated with assisting in minimally invasive surgery. Surg Endosc. 2009;23(1):182–8.
16. van Det MJ, Meijerink WJ, Hoff C, et al. Optimal ergonomics for laparoscopic surgery in minimally invasive surgery suites: a review and guidelines. Surg Endosc. 2009;23(6):1279–85.
17. Sutton E, Youssef Y, Meenaghan N, et al. Gaze disruptions experienced by the laparoscopic operating surgeon. Surg Endosc. 2010;24(6):1240–4.
18. Wiegmann DA, ElBardissi AW, Dearani JA, et al. Disruptions in surgical flow and their relationship to surgical errors: an exploratory investigation. Surgery. 2007;142(5):658–65.
19. Lallas CD, Davis and Members of the Society of Urologic Robotic Surgeons JW. Robotic surgery training with commercially available simulation systems in 2011: a current review and practice pattern survey from the society of urologic robotic surgeons. J Endourol. 2012;26(3):283–93.
20. Abboudi H, Khan MS, Aboumarzouk O, et al. Current status of validation for robotic surgery simulators – a systematic review. BJU Int. 2013;111(2): 194–205.
21. Sunkara SK, Khairy M, El-Toukhy T, et al. The effect of intramural fibroids without uterine cavity involvement on the outcome of IVF treatment: a systematic review and meta-analysis. Hum Reprod. 2010;25(2):418–29.
22. Klatsky PC, Tran ND, Caughey AB, et al. Fibroids and reproductive outcomes: a systematic literature review from conception to delivery. Am J Obstet Gynecol. 2008;198(4):357–66.
23. Shavell VI, Thakur M, Sawant A, et al. Adverse obstetric outcomes associated with sonographically identified large uterine fibroids. Fertil Steril. 2012; 97(1):107–10.
24. Jin C, Hu Y, Chen XC, et al. Laparoscopic versus open myomectomy–a meta-analysis of randomized controlled trials. Eur J Obstet Gynecol Reprod Biol. 2009;145(1):14–21.
25. Bulletti C, Polli V, Negrini V, et al. Adhesion formation after laparoscopic myomectomy. J Am Assoc Gynecol Laparosc. 1996;3(4):533–6.
26. Palomba S, Zupi E, Russo T, et al. A multicenter randomized, controlled study comparing laparoscopic versus minilaparotomic myomectomy: short-term outcomes. Fertil Steril. 2007;88(4):942–51.
27. Takeuchi H, Kinoshita K. Evaluation of adhesion formation after laparoscopic myomectomy by systematic second-look microlaparoscopy. J Am Assoc Gynecol Laparosc. 2002;9(4):442–6.
28. Garnet JD. Uterine rupture during pregnancy. An analysis of 133 patients. Obstet Gynecol. 1964;23: 898–905.
29. Parker WH, Einarsson J, Istre O, et al. Risk factors for uterine rupture after laparoscopic myomectomy. J Minim Invasive Gynecol. 2010;17(5):551–4.
30. Spong CY, Landon MB, Gilbert S, et al. Risk of uterine rupture and adverse perinatal outcome at term after cesarean delivery. Obstet Gynecol. 2007;110(4): 801–7.
31. Pitter MC, Gargiulo AR, Bonaventura LM, et al. Pregnancy outcomes following robot-assisted myomectomy. Hum Reprod. 2013;28(1):99–108.
32. Liu G, Zolis L, Kung R, et al. The laparoscopic myomectomy: a survey of Canadian gynaecologists. J Obstet Gynaecol Can. 2010;32(2):139–48.
33. Advincula AP, Song A, Burke W, et al. Preliminary experience with robot-assisted laparoscopic myomectomy. J Am Assoc Gynecol Laparosc. 2004;11(4): 511–8.

34. Bedient CE, Magrina JF, Noble BN, et al. Comparison of robotic and laparoscopic myomectomy. Am J Obstet Gynecol. 2009;201(6):566.e1–5.
35. Advincula AP, Xu X, Goudeau S, et al. Robot-assisted laparoscopic myomectomy versus abdominal myomectomy: a comparison of short-term surgical outcomes and immediate costs. J Minim Invasive Gynecol. 2007;14(6):698–705.
36. Mais V, Ajossa S, Guerriero S, et al. Laparoscopic versus abdominal myomectomy: a prospective, randomized trial to evaluate benefits in early outcome. Am J Obstet Gynecol. 1996;174(2):654–8.
37. Barakat EE, Bedaiwy MA, Zimberg S, et al. Robotic-assisted, laparoscopic, and abdominal myomectomy: a comparison of surgical outcomes. Obstet Gynecol. 2011;117(2 Pt 1):256–65.
38. Meredith SM, Sanchez-Ramos L, Kaunitz AM. Diagnostic accuracy of transvaginal sonography for the diagnosis of adenomyosis: systematic review and metaanalysis. Am J Obstet Gynecol. 2009;201(1):107.e1–6.
39. Moghadam R, Lathi RB, Shahmohamady B, et al. Predictive value of magnetic resonance imaging in differentiating between leiomyoma and adenomyosis. JSLS. 2006;10(2):216–9.
40. Goto A, Takeuchi S, Sugimura K, et al. Usefulness of Gd-DTPA contrast-enhanced dynamic MRI and serum determination of LDH and its isozymes in the differential diagnosis of leiomyosarcoma from degenerated leiomyoma of the uterus. Int J Gynecol Cancer. 2002;12(4):354–61.
41. Bush AJ, Morris SN, Millham FH, et al. Women's preferences for minimally invasive incisions. J Minim Invasive Gynecol. 2011;18(5):640–3.
42. Gargiulo AR, Bailey AP, Srouji SS. Robot-assisted single-incision laparoscopic myomectomy: initial report and technique. J Robot Surg. 2012. doi: 10.1007/s11701-012-0356-1.
43. Hillis SD, Marchbanks PA, Tylor LR, et al. Poststerilization regret: findings from the United States collaborative review of sterilization. Obstet Gynecol. 1999;93(6):889–95.
44. Roberts DL, Solow AR. Flightless birds: when did the dodo become extinct? Nature. 2003;426(6964):245.
45. Deffieux X, Morin Surroca M, Faivre E, et al. Tubal anastomosis after tubal sterilization: a review. Arch Gynecol Obstet. 2011;283(5):1149–58.
46. Yoon TK, Sung HR, Kang HG, et al. Laparoscopic tubal anastomosis: fertility outcome in 202 cases. Fertil Steril. 1999;72(6):1121–6.
47. Falcone T, Goldberg J, Garcia-Ruiz A, et al. Full robotic assistance for laparoscopic tubal anastomosis: a case report. J Laparoendosc Adv Surg Tech A. 1999;9(1):107–13.
48. Dharia Patel SP, Steinkampf MP, Whitten SJ, Whitten SJ, Malizia BA, et al. Robotic tubal anastomosis: surgical technique and cost effectiveness. Fertil Steril. 2008;90(4):1175–9.
49. Caillet M, Vandromme J, Rozenberg S, Paesmans M, et al. Robotically assisted laparoscopic microsurgical tubal reanastomosis: a retrospective study. Fertil Steril. 2010;94(5):1844–7.
50. Degueldre M, Vandromme J, Huong PT, et al. Robotically assisted laparoscopic microsurgical tubal reanastomosis: a feasibility study. Fertil Steril. 2000;74(5):1020–3.
51. Gargiulo AR. Fertility preservation and the role of robotics. Clin Obstet Gynecol. 2011;54(3):431–48.
52. Practice Committee of the American Society for Reproductive Medicine. Committee opinion: role of tubal surgery in the era of assisted reproductive technology. Fertil Steril. 2012;97(3):539–45.
53. Jacobson TZ, Duffy JM, Barlow D, et al. Laparoscopic surgery for subfertility associated with endometriosis. Cochrane Database Syst Rev. 2010;1:CD001398.
54. Marcoux S, Maheux R, Berube S. Laparoscopic surgery in infertile women with minimal or mild endometriosis. Canadian collaborative group on endometriosis. N Engl J Med. 1997;337(4):217–22.
55. Parazzini F. Ablation of lesions or no treatment in minimal-mild endometriosis in infertile women: a randomized trial. Gruppo Italiano per lo Studio dell'Endometriosi. Hum Reprod. 1999;14(5):1332–4.
56. Practice Committee of the American Society for Reproductive Medicine. Endometriosis and infertility: a committee opinion. Fertil Steril. 2012;98(3):591–8.
57. Celik HG, Dogan E, Okyay E, et al. Effect of laparoscopic excision of endometriomas on ovarian reserve: serial changes in the serum antimullerian hormone levels. Fertil Steril. 2012;97(6):1472–8.
58. Coccia ME, Rizzello F, Mariani G, et al. Ovarian surgery for bilateral endometriomas influences age at menopause. Hum Reprod. 2011;26(11):3000–7.
59. Crosignani PG, Vercellini P, Biffignandi F, et al. Laparoscopy versus laparotomy in conservative surgical treatment for severe endometriosis. Fertil Steril. 1996;66(5):706–11.
60. Kuroda M, Kuroda K, Arakawa A, et al. Histological assessment of impact of ovarian endometrioma and laparoscopic cystectomy on ovarian reserve. J Obstet Gynaecol Res. 2012;38(9):1187–93.
61. Li CZ, Liu B, Wen ZQ, et al. The impact of electrocoagulation on ovarian reserve after laparoscopic excision of ovarian cysts: a prospective clinical study of 191 patients. Fertil Steril. 2009;92(4):1428–35.
62. Barton SE, Gargiulo AR. Robot-assisted laparoscopic myomectomy and adenomyomectomy with a flexible CO_2 laser device. J Robot Surg. 2012. doi: 10.1007/s11701-012-0360-5.
63. Donnez J, Lousse JC, Jadoul P, et al. Laparoscopic management of endometriomas using a combined technique of excisional (cystectomy) and ablative surgery. Fertil Steril. 2010;94(1):28–32.
64. Muzii L, Panici PB. Combined technique of excision and ablation for the surgical treatment of ovarian

endometriomas: the way forward? Reprod Biomed Online. 2010;20(2):300–2.
65. Muzii L, Bellati F, Bianchi A, et al. Laparoscopic stripping of endometriomas: a randomized trial on different surgical techniques. Part II: Pathological results. Hum Reprod. 2005;20(7):1987–92.
66. Muzii L, Bianchi A, Bellati F, et al. Histologic analysis of endometriomas: what the surgeon needs to know. Fertil Steril. 2007;87(2):362–6.
67. Muzii L, Miller CE. The singer, not the song. J Minim Invasive Gynecol. 2011;18(5):666–7.
68. Carvalho L, Abrao MS, Deshpande A, Falcone T, et al. Robotics as a new surgical minimally invasive approach to treatment of endometriosis: a systematic review. Int J Med Robot. 2012;8(2):160–5. doi:10.1002/rcs.451.
69. Nezhat C, Hajhosseini B, King LP. Robotic-assisted laparoscopic treatment of bowel, bladder, and ureteral endometriosis. JSLS. 2011;15(3):387–92.
70. Nezhat C, Lewis M, Kotikela S, Veeraswamy A, et al. Robotic versus standard laparoscopy for the treatment of endometriosis. Fertil Steril. 2010;94(7):2758–60.
71. Bisharah M, Tulandi T. Laparoscopic preservation of ovarian function: an underused procedure. Am J Obstet Gynecol. 2003;188(2):367–70.
72. Morice P, Thiam-Ba R, Castaigne D, et al. Fertility results after ovarian transposition for pelvic malignancies treated by external irradiation or brachytherapy. Hum Reprod. 1998;13(3):660–3.
73. Terenziani M, Piva L, Meazza C, et al. Oophoropexy: a relevant role in preservation of ovarian function after pelvic irradiation. Fertil Steril. 2009;91(3):31.
74. Hwang JH, Yoo HJ, Park SH, et al. Association between the location of transposed ovary and ovarian function in patients with uterine cervical cancer treated with (postoperative or primary) pelvic radiotherapy. Fertil Steril. 2012;97(6):1387–93.e1–2.
75. Barton SE, Politch JA, Benson CB, et al. Transabdominal follicular aspiration for oocyte retrieval in patients with ovaries inaccessible by transvaginal ultrasound. Fertil Steril. 2011;95(5):1773–6.
76. Molpus KL, Wedergren JS, Carlson MA. Robotically assisted endoscopic ovarian transposition. JSLS. 2003;7(1):59–62.
77. Al-Badawi I, Al-Aker M, Tulandi T. Robotic-assisted ovarian transposition before radiation. Surg Technol Int. 2010;19:141–3.
78. Grynberg M, Poulain M, Sebag-Peyrelevade S, le Parco S, et al. Ovarian tissue and follicle transplantation as an option for fertility preservation. Fertil Steril. 2012;97(6):1260–8.
79. Oktay K, Oktem O. Ovarian cryopreservation and transplantation for fertility preservation for medical indications: report of an ongoing experience. Fertil Steril. 2010;93(3):762–8.
80. Mayerhofer K, Ott J, Nouri K, et al. Laparoscopic ovarian tissue harvesting for cryopreservation: an effective and safe procedure for fertility preservation. Eur J Obstet Gynecol Reprod Biol. 2010;152(1):68–72.
81. Donnez J, Jadoul P, Pirard C, et al. Live birth after transplantation of frozen-thawed ovarian tissue after bilateral oophorectomy for benign disease. Fertil Steril. 2012;98(3):720–5.
82. Sonmezer M, Oktay K. Orthotopic and heterotopic ovarian tissue transplantation. Best Pract Res Clin Obstet Gynaecol. 2010;24(1):113–26.
83. Akar ME, Carrillo AJ, Jennell JL, Yalcinkaya TM, et al. Robotic-assisted laparoscopic ovarian tissue transplantation. Fertil Steril. 2011;95(3):1120.e5–8
84. Bedaiwy MA, Falcone T. Whole ovary transplantation. Clin Obstet Gynecol. 2010;53(4):797–803.
85. Silber SJ. Ovary cryopreservation and transplantation for fertility preservation. Mol Hum Reprod. 2012;18(2):59–67.
86. Silber SJ, DeRosa M, Pineda J, et al. A series of monozygotic twins discordant for ovarian failure: ovary transplantation (cortical versus microvascular) and cryopreservation. Hum Reprod. 2008;23(7):1531–7.
87. Silber SJ, Grudzinskas G, Gosden RG. Successful pregnancy after microsurgical transplantation of an intact ovary. N Engl J Med. 2008;359(24):2617–8.
88. Boggi U, Vistoli F, Signori S, et al. Robotic renal transplantation: first European case. Transpl Int. 2011;24(2):213–8. doi:10.1111/j.1432-2277.2010.01191.x.
89. Falk V, Jacobs S, Gummert JF, et al. Computer-enhanced endoscopic coronary artery bypass grafting: the da Vinci experience. Semin Thorac Cardiovasc Surg. 2003;15(2):104–11.
90. Semm K, Mettler L. Technical progress in pelvic surgery via operative laparoscopy. Am J Obstet Gynecol. 1980;138(2):121–7.
91. AAGL Advancing Minimally Invasive Gynecology Worldwide. AAGL position statement: robotic-assisted laparoscopic surgery in benign gynecology. J Minim Invasive Gynecol. 2013;20(1):2–9.

Part III

Assisted Reproduction Techniques

Advances in Systems for Embryo Culture

9

Roberta Maggiulli, Lisa Dovere, Filippo Ubaldi, and Laura Rienzi

9.1 Introduction

In the last decade, several efforts have pointed to a better awareness of the embryo physiology and biochemistry, leading to significant advances in systems for embryo culture.

It is well known that a short in vitro culture does not allow for a reliable embryo evaluation, requiring the transfer of more than one embryo and thus increasing the risk for multiple pregnancies. However, only up to half of human embryos conceived in vitro develop to the blastocyst stage and ~18 % of them arrest at or prior to the 4-cell stage [1, 2]. Beyond the substantial genetic defects that are intrinsic to the embryos, suboptimal culture media composition or physical culture parameters (or a combination of them) may be responsible for these observed rates of development arrest.

The preimplantation embryo is a free-living organism that can regulate its own cell division and differentiation using transcripts accumulated during oogenesis [3] and produced after the activation of the embryonic genome [4]. Additionally, this autonomous organism produces embryotrophic factors such as platelet-activating factor and interleukin-1 regulating the early events of embryo development [5]. The first crucial steps of mammalian development such as first cleavage, activation of maternal genome, compaction, and differentiation are the result of precisely programmed and orchestrated events. The embryo is also endowed with the ability to adapt to changing environmental conditions that maintains cellular homeostasis and preserves viability. Despite this embryonic plasticity, the exposure to suboptimal environmental conditions that can exceed its adaptive capacity may cause change in epigenetics, transcription, metabolism, and cell allocation with potential long-term consequences [6, 7].

In the recent years, many researches pointed to improve embryo culture conditions and to introduce novel devices and platforms to provide a more appropriate microenvironment for the embryos. The majority of acquired knowledge has led to enrich media formulation, refining them by introducing salts, amino acids, energy substrates, growth factors, and other supplements. Overall, these advances have made feasible to extend embryo culture to the blastocyst stage, allowing single embryo transfer while accomplishing consistent pregnancy and live birth rates, thus increasing significantly the efficiency of human-assisted reproduction procedures.

However, not only the chemical supplies of the developing embryo need to be considered but also potential physical requirements (mechanical and surface interactions, cell movement) may influence embryo development and may be important factors in the continuing pursuit of

R. Maggiulli, B.S. • L. Dovere, B.S.
F. Ubaldi, M.D., M.Sc. • L. Rienzi, M.Sc. (✉)
GENERA Centre for Reproductive Medicine,
Clinica Valle Giulia, Rome, Italy
e-mail: rienilaura@gmail.com

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_9,

improved in vitro conditions. To this end, very recently novel culture and surface platforms have been developed, allowing dynamic culture through the employment of media flows.

Despite several aspects remain to be analyzed, these new approaches and emerging technologies may optimize the efficiency of embryo production, creating a more appropriate microenvironment for gamete function and support embryo developmental competence.

9.2 Embryo Culture Platforms

9.2.1 Static Culture Platforms

Until now, human embryos have been commonly cultured on inert plastic supports that create a "static microenvironment" as these platforms do not produce any active movements and limited cell surface contact [8]. During routine IVF procedure, culture media are conventionally placed in disposable polystyrene multiwell or Petri dishes, in 10–80 μl drops of media covered with oil and equilibrated overnight in the proper gas mixture at 37 °C to stabilize the pH, temperature, and achieve proper gas saturation. Generally, the embryos are cultured individually or in small groups and incubated for days, in either single or sequential medium [7, 9, 10]. However, in vivo, embryos are exposed to a more dynamic environment, developing in the virtual space of oviduct. As previously underlined [9], considerable differences exist between the conventional culture system and the natural environment of the oviduct. The female reproductive tract is surrounded by ciliated epithelia that sustain embryo movement; moreover, during this progression embryos are exposed to several unknown constituents of oviductal fluids that fulfill the metabolic needs of the embryo.

This is in sharp contrast with the in vitro environment, where embryos are cultured on artificial surface and no dynamic movements are ensured and where autocrine factors are often diluted and diffused into the oil layer.

9.2.2 Enhanced Static Platforms

Recently, novel devices and new culture approaches are being developed in order to handle physical parameters and to improve the in vitro microenvironment, exploiting different potential beneficial aspects of embryo culture such as increased embryo density, decreased media volume, and retention of autocrine/paracrine factors.

Embryo density, expressed as the embryo-to-volume ratio, is the number of embryos in a defined volume of culture medium. The same density can be achieved by manipulating either the number of embryos in a given volume of medium, or manipulating the volume of the medium for a given number of embryos. In different animal models, it has been observed that increased embryo density may improve developmental competence, probably through the production and secretion of various factors able to affect embryo homeostasis [11–14]. Recently it has been shown that group culture improves rates of human blastocyst development, when compared to individual culture [15, 16].

Embryo culture may be successfully performed in small volume to effectively benefit of retention of autocrine factors. In fact, the mixture of compounds embryo-secreted is challenging to be replaced by exogenous biomolecules.

Moreover, utilization of exogenous growth factors may be inadvisable since an appropriate spatial or temporal exposure may lead to developmental abnormalities such as large offspring syndrome [17, 18].

In order to confine embryos to a small area, microdrop systems have long been used. Generally, these drops varied from 10 to 50 μl of volume and can be used with group or individual embryo culture, although most embryologists prefer individual culture for easily identification and follow up. A limitation of this approach is related to the potential drop flattening or coalescing, entailing a variation in the amount of media where embryos are cultured and hampering the embryo tracking during handling and evaluation. Specialized dishes are now available, specifically

designed for embryo culture and employing small round bottom wells inside a traditional Petri dish that allows for retention of putative embryotrophic factors while preserving the individuality of each single embryo.

Another variation of this approach utilizes ultralow volumes of media, the "ultramicrodrop system". The volume of these drops ranges from 1.5 to 2 μl and allows to culture and confine groups of embryos in a small area and to concentrate autocrine/paracrine factors. This approach has resulted in improved embryo development, although tested only with very few embryos. However, further and detailed analysis including pregnancy and implantation rates are necessary to investigate the potential risk of using very small volumes of media, where rapid evaporation with dangerous increase in osmolality can occur [8].

New culture platforms have been developed utilizing extremely low volume of media with a limited surface area. The submicroliters platforms are composed of a culture chip of polydimethylsiloxane (PDMS) containing a small vertical channel. During the culture, 2-cell embryos in the vertical channel are surrounded by submicroliters volume (100 nl) [19]. Rates of blastocyst development obtained using this culture system were comparable with 20-μl culture systems, but significantly greater than 5-μl microdrop cultures. Thus, this novel device allows embryos to take advantages from reduced culture volume and spacing while avoiding issues correlated with small microdrop volume; however, it is limited by a complicate embryo recovery [8].

A novel solution is represented by the Well-of-the-Well (WOW) system, a culture device where embryos are confined in small area while sharing a larger reservoir of media. Basically, it consists of small microwells of conical shape created inside of a well of a 4-well dish or in a Petri dish. First described by Vajta [14], this approach has been successfully used with embryos from several species such as mouse, pig, and human. The advantage of this system is that embryos can be cultured individually in each microwell while sharing the same overlying medium; this creates a microenvironment around the embryos, increasing the point of contact between them. According to an initial human trial, higher blastocyst rates were observed when embryos were cultured in WOW devices compared to microdrop system (56 vs. 37 %) [20]. Although this system appears very promising, data regarding pregnancy and birth rates are still preliminary and further investigations are required.

The "glass oviduct (GO) system" was proposed by Thouas et al. in 2003 [12] as alternative solution. This culture system is composed of 2-μl sterile open-ended capillary with 200-μm inner diameter. Embryos are loaded by immersing one end of the capillary in a standard microdrop system. Initially, a small oil column enters into the glass capillary, followed by the medium with the embryos, finally upon retraction, oil enters again into the column and closes the solution. Then the capillary is cultured in vertical position in a carbon dioxide incubator and the medium surrounding embryos is approximately 1 μl; this allows creating concentration gradients for several factors selected or discarded by the embryos. Although blastocyst rates obtained in mouse model were similar to those achieved by traditional culture methods, culturing embryos in the GO system has allowed to improve others parameters such as blastocyst total cell number and hatching rates [21].

The GO system can be considered as an extremely simplified and static version of the microchannel system. More sophisticated and purpose-designed versions of microchannels have been regarded as the greatest promise to establish a multipurpose automated system for in vitro production of preimplantation embryos.

9.2.2.1 Specialized Surface Coating

Enhancing culture conditions entails also the revision of the surface of the devices where embryos are cultured.

Several synthetic polymers have been tested on mouse embryos to investigate the potential toxicity due to contaminants or different additives. Generally, conventional devices are made by

polystyrene and glass, materials that are heat-stable and tolerate the temperature and humidity of the incubator without interfering with media [8]. The use of PMDS as IVF device is particularly critical, since it could modify media composition or cause detrimental osmolality shifts [22, 23]. However, the static inert devices used for embryo culture are extremely different from the dynamic interactive surfaces to which embryos are exposed in the uterine cavity. In vivo, embryos are surrounded by several macromolecules and components of extracellular matrix that are thought to support embryo cellular homeostatic mechanisms, imparting responsiveness or plasticity to the embryo [24–26]. These macromolecules are supposed to act in a physical sense to stabilize the chemical environments along the oviduct, interacting with biological fluids and inducing significant modifications of the fluid surrounding the embryos. The inclusion of constituents, such as glycosaminoglycans, could improve embryo culture, altering surface properties such as hydrophilicity and aiming to reproduce more closely the female reproductive tract. Equally, glycoproteins are believed to act as carrier molecules to present cations and metabolic substrates at appropriate concentration to the embryo [24].

Figueiredo and collaborators found that laminin added to culture media was detrimental to embryo development decreasing cell number in mouse blastocyst, whereas fibronectin was compatible with mouse embryo development, even if no positive effect was observed compared to controls. Other investigations found that fibronectin and laminin could improve human blastocyst hatching rates if used at 50 μg/ml [27], underlying the different species-specific actions and the importance of concentration. Also, Heparin, hyaluronic acid, and chondroitin sulfate have been added to culture media improving blastocyst development in bovine embryos [28]. These macromolecules can act as anchor for different growth factors, thus their proper orientation is important to influence embryo development. It has been demonstrated that the employment of matrigel (a solubilized basement membrane preparation, rich in Ecm protein) as plate coating increased rate of mouse blastocyst hatching at 96 and 120 h, even if other authors have shown a detrimental effect of the same coating on mouse blastocyst development, probably due to a different mouse strain used [29, 30].

Conflicting data exist regarding the use of hyaluronic acid, since after preliminary encouraging results in mouse and bovine models, the use of hyaluronic-coated culture surface has significantly reduced mouse blastocyst cell number [31]. A different approach utilizing agarose-made microwells did not display any benefits during embryo culture.

In 1965, Cole and Paule [32], in the attempt to more closely mimic the in vivo microenvironment, provided the proof of concept that mouse embryos could benefit from coculture with somatic cells. The use of feeder cell lines was then investigated in human in vitro culture, with conflicting clinical results. Initial studies in human IVF, using bovine uterine epithelial cells and human oviductal cells [33], showed promising results and led to a great deal of optimism that coculture may improve embryo development. The observed benefits in terms of improved embryo quality were due both to the secretion of embryotrophic factors and the detoxification of the culture medium [34–37]. Limited studies in this field have been performed; a systematic review of randomized controlled trials was performed by Kattal et al. [38] in order to objectively determine the potential benefits of coculture in human IVF, revealing a statistically significant improvement in embryo morphology and clinical outcome when coculture is performed.

However, the use of biological materials has been complicated by the potential risk of contamination or transmission of disease from feeder layers to the developing embryos. As a consequence of the limitations introduced by the U.S. Food and Drug Administration in 2002 (limiting the use of nonhuman coculture cell lines for human IVF), autologous endometrial cells have been introduced for coculture.

Currently, there is still a lack of information regarding these novel culture platforms and human embryos. Although several proteoglycans and oviductal-specific proteins have been identified, the comprehension of the real impact

of these biomolecules on human embryo development requires more exhaustive studies. Because of these controversial results, the use of coated platforms is not as widespread as expected, yet.

9.2.3 Dynamic Culture Platforms

As discussed above, static embryo culture has been the mainly employed method so far. Although these platforms are not completely static, because of convection currents and movement of dishes that can shake media during the observations, they are not fully proper to satisfy the ever-changing needs of preimplantation embryos. In vivo, embryos are exposed to a dynamic and gradually changing microenvironment sustained by peristaltic contraction of the smooth muscle of the fallopian tube and kinetic friction forces with ciliated epithelia. During its journey alongside the reproductive tract, the embryo is exposed to constant vibrations of around 6 Hz with the periodically repeating increase to 20 Hz that stimulate embryonic mechanoreceptors and induce the cell-to-cell communication [39].

Conversely, conventional static embryo culture systems require several washing and changing of media during the preimplantation period and expose embryos to suboptimal atmosphere and sudden changes in microenvironment conditions. Furthermore, the accumulation of toxic substances, such as oxygen-derived radicals [40] and ammonia [41], may have a detrimental effect on embryo development. Moreover, studies monitoring the mouse embryo physiology have measured gradients of potassium, calcium, and oxygen around unperturbed embryos [42], due to the secretion or depletion of media components by the developing embryo.

Therefore, new dynamic platforms, specifically assembled in order to produce flow of media, have been proposed to disrupt these gradients and to create a more homogenous environment around the embryo, thus mimicking closely the in vivo conditions.

Although providing mechanical stimuli may improve embryo developmental ability, several limitations characterize these promising novel culture systems; first of all their complexity and lab compatibility with respect to static culture platforms. Besides biocompatibility, that is of utmost importance, other factors such as friction and flow rate have to be carefully considered. Excess mechanical stimuli or overhandling of embryos can induce transient changes in embryo homeostasis and significantly impair embryo viability [43, 44]. Moreover, a continuous rough refreshment of medium may lead also to the elimination of beneficial auto- and paracrine factors [45].

To sum up, there are different hypotheses that explain the potential benefits of dynamic culture systems: the gentle agitation of media that remove waste products around the embryos with replenishment of fresh substrates, the disruption of environmental gradient and the physical stimulation able to activate mechanoreceptors, or signaling pathways involved in embryo development. Unfortunately, not all dynamic culture platforms can have all the characteristics mentioned above; thus, several approaches to generate dynamic culture have been examined.

One of the first approaches to perform a dynamic embryo culture is the use of an orbital shaker placed inside the incubator [46]. Using this culture system, embryos were agitated at 60 rev/min, cultured in a volume of 0.5 ml overlaid with oil. The first promising results came from mouse embryos and ovarian tissue culture [47]. Higher rates of blastocyst development (98.5 %) have been obtained using orbital rotation on flat surface with respect to static culture platform (86.3 %) [31].

While different volumes of media and times of agitation do not have a significant effect on embryo viability, instead the rate of rotation seems to have an impact on embryo development, having detrimental effect when rates of orbital movement arrived at 60 rev/min [8].

Another easy-to-implement alternative to conventional static platforms is represented by the tilting embryo culture system (TECS). A motorized tilting platform is composed of a control unit to set the speed, the angle, and the period of tilting and of a motor unit to drive stage tilting and to place conventional culture dish. While embryos

are tilted, the rolling and media agitation try to mimic the movement through the reproductive tract. Mouse and human embryos have been successfully cultured in TECS, showing an enhanced cell division and blastocyst quality compared to controls [48]. A benefit of this system relies on its lab compatibility that may allow a widespread utilization; however, additional clinical investigations are required to analyze the potential benefits and the limitation of TECS.

To induce dynamic culture conditions, a simple vibration may be sufficient. Initially, pulsatile mechanical microvibration has been successfully used to mature porcine oocytes improving developmental competence and subsequently embryo growth [49]. Also, human zygotes were cultured using gentle vibration of 20 Hz for 5 s [50]. Although the introduction of microvibration did not influence fertilization rates compared to static controls, a significantly higher percentage of high-quality cleavage stage embryos was observed compared with static culture system (90.1 vs. 77.9 %, $p<0.05$). Moreover, the percentage of embryos that reached the blastocyst stage was 10 % higher than that recorded for the static culture system. This enhanced in vitro embryo development in vitro resulted in a significantly higher pregnancy rate regardless of the day of embryo transfer, highlighting the benefits of gentle vibration during embryo culture.

9.3 Microchannel Microfluidic System

The replenishment of culture media and removal of harmful factors produced by embryos is not accomplished with novel culture devices mentioned above. Moreover, embryos are exposed to suboptimal conditions during handling and the great amount of media to which are exposed may temper the presence of embryotrophic factor.

The great advantage of microfluidic system is that all the requisites to obtain an optimized embryo culture can be accomplished at once. This system allowed performing culture of embryos in precisely defined, submicroliter volumes minimizing the risk of evaporation and to maintain the surface area-to-volume (SA/V) ratios in a physiological range. The spacing theory is already supported by several investigations demonstrating that improved embryo development can be achieved using ultramicrodrops [51], glass capillary tubes [12, 13], and WOW technologies [14].

Others benefits may arise from the gradual replenishment of media around the embryo and from the mechanical induction of cellular pathways involved in embryo development.

The microchannel microfluidic system is not a recent technique since it has been developed during the 1980s of the last century with multidisciplinary purpose and applications in different fields from physics and chemistry to micro- and biotechnology. The approach of dynamic media flow obtained with microfluidic platforms varied greatly in design and are used for various aspect of ART such as in vitro oocyte maturation [52, 53] and sperm selection [54, 55] and recently also as platform for embryo culture [56, 57].

The microchannel system is essentially composed of the following parts: a glass microscopic slide base and plastic layer with the channels and valves connected with automatic or mechanical pumps.

A critical aspect of microfluidic system is the flow rate that must be finely regulated to determine the range for beneficial effects, since shear stress can influence negatively embryo development, causing damage to blastomeres and embryo degeneration [44].

The early devices used in ART employed passive flow driven by gravity; others used manually applied pressure created by syringes connected externally or programmable syringe infusion pump [55, 58–60]. However, these approaches require constant and difficult regulation of the flow; thus, they are not of easy employment [61]. Very recently, a new Braille pumping system using electric piezo actuators has been successfully introduced, aiming to create a peristaltic movement of media along microchannels. This system assures computerized regulation of the flow without constant supervision and allows gradual variation of media flowing toward the embryos [61].

Glasgow and coauthors are first to demonstrate that embryo manipulation and movement in a microfluidic system is feasible at low flow rates without injuring the embryos [62]. Once the safety of microfluidic system was proved, some authors [56] have shown that 2-cell mouse embryos could be cultured using microchannel system with submicroliter culture volume, with significantly higher blastocyst rate at 48 h and at 72 h (17.6 vs. 2.4 % and 72.9 vs. 42.9 %, respectively) and hatching blastocyst rate at 72 h and 96 h (4.1 vs. 0 % and 26.5 vs. 8.8 %, respectively). Although the effective volume surrounding the embryos was 250 μl, the employment of very tiny channels (from 100 nm to several hundred micrometers) avoids the occurrence of turbulence and maintains a laminar flow. This microfluidic system, however, has not yet been shown to enhance pregnancy rates. Heo and coworkers established a dynamic microfunnel embryo culture system to better mimicking the fluid-mechanical and biochemical stimulation that embryo experienced in vivo [63]. Blastocyst developmental rate was significantly enhanced under dynamic microfunnel culture conditions as evidenced by an increased percentage of hatching or hatched blastocysts and significantly higher average number of cells per blastocyst. Most importantly, preimplantation developmental kinetics and clinical performances of embryos developed in dynamic conditions more closely resemble those of the in vivo counterparts. Compared to microchannel culture, dynamic microfunnel system allows to benefit either from fluid mechanical stimulation to the embryo or from retention of a significant amount of embryotrophic factors simultaneously.

These encouraging data, although preliminary, indicate that the microfluidic technology has great potential for improving clinical ART and may represent a solution to meet the mutable needs of embryos, while maintaining an optimal microenvironment during the preimplantation culture.

Taken together, these novel approaches could potentially revolutionize the concept of embryo culture; unfortunately, most of the data discussed above arise from animal models, whereas there are little evidences that these approaches truly benefit also human embryos. Moreover, the implementation of the IVF laboratories with these new technologies would require significant economical efforts.

9.4 Integrated Automated System for Embryo Production

Once established, the enhanced culture system can also be integrated with other equipment as a video camera to monitor all steps of the embryo development (Fig. 9.1). Such purpose-designed

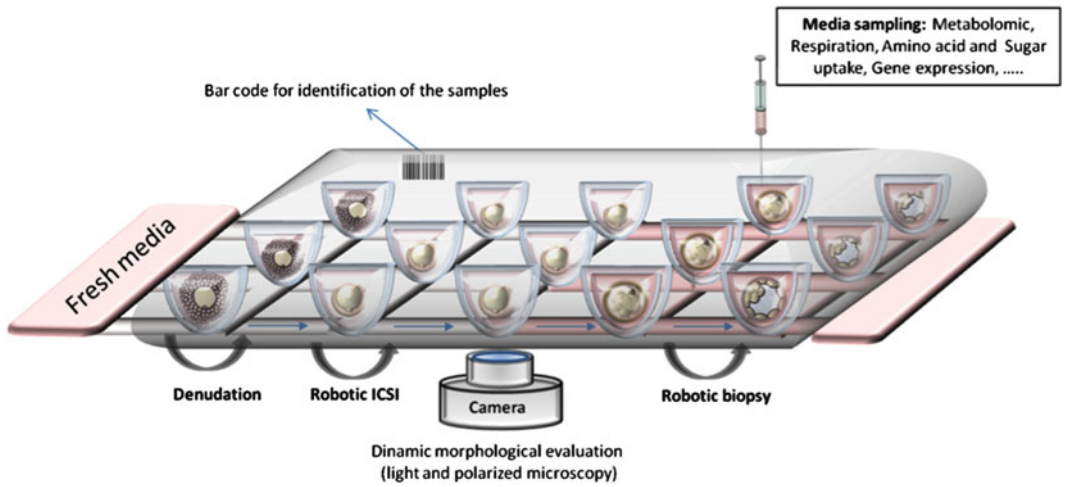

Fig. 9.1 Integrated automated system for embryo production

instruments are already available and provided their value in embryo selection [64, 65]. Further extensions may include various sensors measuring parameters such as embryo-derived biomarkers (metabolomics) or gene expression profiles (transcriptomics). The enormous amount of information derived from the time-lapse imaging together with the biochemical parameters may provide a significant support to select the best embryo(s) to transfer and to compare the efficiency various culture methods.

Eventually, the microchannel system may also be useful to personalize embryo culture according to the individual needs of each embryo to compensate deviations in metabolism [9]. However, caution is suggested while using this approach. It should be considered that embryos are autonomous living beings with proven ability to establish their proper microenvironment even under compromised conditions. On the other hand, their adaptation ability to the ever-changing environment may be limited, and continuous or frequently repeated flushing even with the most sophisticated solutions may cause more problems than benefits. A proper use of the enormous possibilities offered by the microchannel system may help to find the right compromise and to bridge the existing gap between the technology level of laboratory embryology and that of other prominent branches of science.

An ideal system should also reduce risk of mistakes providing secure identification of the biological material during each stage of a patient's cycle. Measures, such as labeling of all lab ware and double-witnessing protocols, are currently employed in IVF laboratory worldwide. Recently, innovative solutions for electronic witnessing that allow automatic recognition and confirmation of sample identity and matching have been developed as an alternative to double witnessing (Fig. 9.2). This is already possible by using Radio Frequency Identification (RFID) technology to track and record patient samples monitoring all critical steps carried out in the laboratory (RI Witness™ Research Instruments, UK). In future, direct tagging of embryos through the microinjection of silicon-based barcodes in the perivitelline space could be considered to minimize mismatching errors during ART procedures.

Fig. 9.2 Electronic witnessing using Radio Frequency Identification (RFID) technology

References

1. Behr B. Blastocyst culture and transfer. Hum Reprod. 1999;14(1):5–6.
2. Jun SH, Choi B, Shahine L, et al. Defining human embryo phenotypes by cohort-specific prognostic factors. PLoS One. 2008;3(7):e2562.
3. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature. 1988; 332(6163):459–61.
4. Schultz RM. From egg to embryo: a peripatetic journey. Reproduction. 2005;130(6):825–8.
5. Gopichandran N, Leese HJ. The effect of paracrine/autocrine interactions on the in vitro culture of bovine preimplantation embryos. Reproduction. 2006;131(2): 269–77.
6. Lonergan P, Fair T, Corcoran D, et al. Effect of culture environment on gene expression and developmental characteristics in IVF-derived embryos. Theriogenology. 2006;65(1):137–52.
7. Vajta G, Rienzi L, Cobo A, et al. Embryo culture: can we perform better than nature? Reprod Biomed Online. 2010;20(4):453–69.

8. Swain JE, Smith GD. Advances in embryo culture platforms: novel approaches to improve preimplantation embryo development through modifications of the microenvironment. Hum Reprod Update. 2011;17(4): 541–57.
9. Rienzi L, Vajta G, Ubaldi F. New culture devices in ART. Placenta. 2011;32 Suppl 3:S248–51.
10. Maggiuli R, Ubaldi F, Rienzi L. Oocyte insemination and culture. In: Ginsburg ES, Racowsky C, editors. In vitro fertilization: a comprehensive guide. New York: Springer; 2012. p. 83–98.
11. Richter KS. The importance of growth factors for preimplantation embryo development and in-vitro culture. Curr Opin Obstet Gynecol. 2008;20(3):292–304.
12. Thouas GA, Jones GM, Trounson AO. The 'GO' system–a novel method of microculture for in vitro development of mouse zygotes to the blastocyst stage. Reproduction. 2003;126(2):161–9.
13. Lane M, Gardner DK. Effect of incubation volume and embryo density on the development and viability of mouse embryos in vitro. Hum Reprod. 1992; 7(4):558–62.
14. Vajta G, Peura TT, Holm P, et al. New method for culture of zona-included or zona-free embryos: the Well of the Well (WOW) system. Mol Reprod Dev. 2000; 55(3):256–64.
15. Ebner T, Shebl O, Moser M, et al. Group culture of human zygotes is superior to individual culture in terms of blastulation, implantation and life birth. Reprod Biomed Online. 2010;21(6):762–8.
16. Rebollar-Lazaro I, Matson P. The culture of human cleavage stage embryos alone or in groups: effect upon blastocyst utilization rates and implantation. Reprod Biol. 2010;10(3):227–34.
17. Wang W, Liu X, Gelinas D, et al. A fully automated robotic system for microinjection of zebrafish embryos. PLoS One. 2007;2(9):e862.
18. Machtinger R, Racowsky C. Morphological systems of human embryo assessment and clinical evidence. Reprod Biomed Online. 2013 26(3):210–21.
19. Melin J, Lee A, Foygel K, et al. In vitro embryo culture in defined, sub-microliter volumes. Dev Dyn. 2009;238(4):950–5.
20. Vajta G, Korosi T, Du Y, et al. The Well-of-the-Well system: an efficient approach to improve embryo development. Reprod Biomed Online. 2008;17(1): 73–81.
21. Vajta G, Lewis IM, Hyttel P, et al. Somatic cell cloning without micromanipulators. Cloning. 2001;3(2):89–95.
22. Toepke MW, Beebe DJ. PDMS absorption of small molecules and consequences in microfluidic applications. Lab Chip. 2006;6(12):1484–6.
23. Heo YS, Cabrera LM, Song JW, et al. Characterization and resolution of evaporation-mediated osmolality shifts that constrain microfluidic cell culture in poly(dimethylsiloxane) devices. Anal Chem. 2007; 79(3):1126–34.
24. Hunter RH. Modulation of gamete and embryonic microenvironments by oviduct glycoproteins. Mol Reprod Dev. 1994;39(2):176–81.
25. Pool TB, Martin JE. High continuing pregnancy rates after in vitro fertilization-embryo transfer using medium supplemented with a plasma protein fraction containing alpha- and beta-globulins. Fertil Steril. 1994;61(4):714–9.
26. Pool TB. Recent advances in the production of viable human embryos in vitro. Reprod Biomed Online. 2002;4(3):294–302.
27. Turpeenniemi-Hujanen T, Feinberg RF, Kauppila A, et al. Extracellular matrix interactions in early human embryos: implications for normal implantation events. Fertil Steril. 1995;64(1):132–8.
28. Jang G, Lee BC, Kang SK, et al. Effect of glycosaminoglycans on the preimplantation development of embryos derived from in vitro fertilization and somatic cell nuclear transfer. Reprod Fertil Dev. 2003; 15(3):179–85.
29. Carnegie J, Claman P, Lawrence C, et al. Can Matrigel substitute for Vero cells in promoting the in-vitro development of mouse embryos? Hum Reprod. 1995;10(3):636–41.
30. Dawson KM, Baltz JM, Claman P. Culture with Matrigel inhibits development of mouse zygotes. J Assist Reprod Genet. 1997;14(9):543–8.
31. Oakes M, Cabrera L, Nanadivada H, et al., editors. Effect of 3-dimensional topography, dynamic fluid movement and an insoluble glycoprotein matrix on murine embryo development. SGI annual meeting, Glasgow, Scotland, 2009.
32. Cole RJ, Edwards RG, Paul J. Cytodifferentiation in cell colonies and cell strains derived from cleaving ova and blastocysts of the rabbit. Exp Cell Res. 1965;37:501–4.
33. Wiemer KE, Cohen J, Amborski GF, et al. In-vitro development and implantation of human embryos following culture on fetal bovine uterine fibroblast cells. Hum Reprod. 1989;4(5):595–600.
34. Barmat LI, Worrilow KC, Paynton BV. Growth factor expression by human oviduct and buffalo rat liver coculture cells. Fertil Steril. 1997;67(4):775–9.
35. Pampfer S, Arceci RJ, Pollard JW. Role of colony stimulating factor-1 (CSF-1) and other lymphohematopoietic growth factors in mouse pre-implantation development. BioEssays. 1991;13(10):535–40.
36. Loutradis D, John D, Kiessling AA. Hypoxanthine causes a 2-cell block in random-bred mouse embryos. Biol Reprod. 1987;37(2):311–6.
37. Fukui Y, McGowan LT, James RW, et al. Factors affecting the in-vitro development to blastocysts of bovine oocytes matured and fertilized in vitro. J Reprod Fertil. 1991;92(1):125–31.
38. Kattal N, Cohen J, Barmat LI. Role of coculture in human in vitro fertilization: a meta-analysis. Fertil Steril. 2008;90(4):1069–76.
39. Isachenko E, Maettner R, Isachenko V, et al. Mechanical agitation during the in vitro culture of human preimplantation embryos drastically increases the pregnancy rate. Clin Lab. 2010;56(11–12):569–76.
40. Johnson MH, Nasr-Esfahani MH. Radical solutions and cultural problems: could free oxygen radicals be

responsible for the impaired development of preimplantation mammalian embryos in vitro? BioEssays. 1994;16(1):31–8.
41. Gardner DK, Lane M. Amino acids and ammonium regulate mouse embryo development in culture. Biol Reprod. 1993;48(2):377–85.
42. Trimarchi JR, Liu L, Smith PJ, et al. Noninvasive measurement of potassium efflux as an early indicator of cell death in mouse embryos. Biol Reprod. 2000; 63(3):851–7.
43. Xie Y, Wang F, Zhong W, et al. Shear stress induces preimplantation embryo death that is delayed by the zona pellucida and associated with stress-activated protein kinase-mediated apoptosis. Biol Reprod. 2006;75(1):45–55.
44. Xie Y, Wang F, Puscheck EE, et al. Pipetting causes shear stress and elevation of phosphorylated stress-activated protein kinase/jun kinase in preimplantation embryos. Mol Reprod Dev. 2007;74(10):1287–94.
45. Fukui Y, Lee ES, Araki N. Effect of medium renewal during culture in two different culture systems on development to blastocysts from in vitro produced early bovine embryos. J Anim Sci. 1996;74(11):2752–8.
46. Hoppe PC, Pitts S. Fertilization in vitro and development of mouse ova. Biol Reprod. 1973;8(4):420–6.
47. Isachenko V, Montag M, Isachenko E, et al. Effective method for in-vitro culture of cryopreserved human ovarian tissue. Reprod Biomed Online. 2006;13(2): 228–34.
48. Matsuura K, Hayashi N, Kuroda Y, et al. Improved development of mouse and human embryos using a tilting embryo culture system. Reprod Biomed Online. 2010;20(3):358–64.
49. Mizobe Y, Yoshida M, Miyoshi K. Enhancement of cytoplasmic maturation of in vitro-matured pig oocytes by mechanical vibration. J Reprod Dev. 2010; 56(2):285–90.
50. Isachenko V, Maettner R, Sterzik K, et al. In-vitro culture of human embryos with mechanical microvibration increases implantation rates. Reprod Biomed Online. 2011;22(6):536–44.
51. Ali J. Continuous ultra-microdrop culture yields higher pregnancy and implantation rates than either large-drop culture or fresh-medium replacement. J Clin Embryol. 2004;7:1–23.
52. Walters EM, Beebe DJ, Wheeler MB. In vitro maturation of pig oocytes in PDMS and silicon microfludic devices. Theriogenology. 2001;55(1):497.
53. Hester PN, Roseman HM, Clark SG, et al. Enhanced cleavage rates following in vitro maturation of pig oocytes within polydimethylsiloxane-borosilcate microchannels. Theriogenology. 2002;57(1):723.
54. Cho BS, Schuster TG, Zhu X, et al. Passively driven integrated microfluidic system for separation of motile sperm. Anal Chem. 2003;75(7):1671–5.
55. Shibata D, Ando H, Lwase A, et al. Analysis of sperm motility and fertilization rates after the separation by microfluidic sperm sorter made of quartz. Fertil Steril. 2007;88(Supp 1):S110.
56. Raty S, Walters EM, Davis J, et al. Embryonic development in the mouse is enhanced via microchannel culture. Lab Chip. 2004;4(3):186–90.
57. Nakamura H, Mizuno J, Akaishi K, et al., editors. New embryo co-culture system for human assisted reproductive technology (ART) by OptiCell. 23rd Annual meeting of the ESHRE, Lyon, France, 2007.
58. Schuster TG, Cho B, Keller LM, et al. Isolation of motile spermatozoa from semen samples using microfluidics. Reprod Biomed Online. 2003;7(1): 75–81.
59. Clark SG, Haubert K, Beebe DJ, et al. Reduction of polyspermic penetration using biomimetic microfluidic technology during in vitro fertilization. Lab Chip. 2005;5(11):1229–32.
60. Hickman DL, Beebe DJ, Rodriguez-Zas SL, et al. Comparison of static and dynamic medium environments for culturing of pre-implantation mouse embryos. Comp Med. 2002;52(2):122–6.
61. Swain J, Pool TB, Takayama S, et al. Microfluidics in ART: time to go with the flow? J Clin Emrbyol. 2008;11(2):5–18.
62. Glasgow IK, Zeringue HC, Beebe DJ, et al. Handling individual mammalian embryos using microfluidics. IEEE Trans Biomed Eng. 2001;48(5): 570–8.
63. Heo YS, Cabrera LM, Bormann CL, et al. Dynamic microfunnel culture enhances mouse embryo development and pregnancy rates. Hum Reprod. 2010; 25(3):613–22.
64. Meseguer M, Herrero J, Tejera A, et al. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod. 2011;26(10):2658–71.
65. Kirkegaard K, Agerholm IE, Ingerslev HJ. Time-lapse monitoring as a tool for clinical embryo assessment. Hum Reprod. 2012;27(5):1277–85.

Patient-Tailored Approaches to Ovarian Stimulation in ART

10

Theodora C. van Tilborg, Frank J.M. Broekmans, Helen L. Torrance, and Bart C. Fauser

10.1 Introduction to Assisted Reproductive Technology

The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) have defined infertility as a disease of the reproductive system by failure to achieve a clinical pregnancy after at least 12 months of regular unprotected sexual intercourse [1]. Of couples trying to conceive, 85–90 % conceives spontaneously within 12 months with most pregnancies occurring within the first 6 months [2]. Approximately 10–17 % of all couples need specialised fertility care once in their lives [2, 3].

Interventions to improve chances of a live birth for subfertile couples consist of fertility enhancing drug therapy, tubal, ovarian and uterine surgery or procedures such as intrauterine insemination (IUI) or in vitro fertilisation (IVF), where the latter is considered to be the treatment of last resort. IVF treatment consists of controlled ovarian stimulation to create multifollicular growth (COS), ovum pickup, in vitro fertilisation, embryo selection and embryo transfer. Medication used for ovarian stimulation for IVF has evolved from clomiphene citrate (CC), human menopausal gonadotropins (hMG), purified urinary follicle stimulating hormone (uFSH) to human recombinant FSH (rFSH). Recently, the efficacy and safety of a long-acting rFSH agonist has also been established [4, 5]. Today, gonadotropins are the principal agents for COS with starting doses varying between 100 and 600 IU/day [6]. Midcycle dose adjustments depending on the ovarian response are often performed despite the fact that solid evidence confirming positive effects of these dose adjustments is still lacking [5, 7].

Over the years, additional interventions have been developed to optimise IVF, including gonadotropin releasing hormone (GnRH) analogue co-treatment to reduce the chance of spontaneous ovulation during COS and human chorionic gonadotropin (hCG) administration before ovum pickup in order to increase the amount of mature oocytes [5]. In current practice, conventional maximal stimulation protocols, using GnRH agonists in a long suppression scheme, with high dosages of FSH, are still the standard treatment, based on the view that "more is better". Mild ovarian stimulation, using the spontaneous cycle as starting point, has focussed on a more moderate ovarian response. It aims to reduce side effects, complications [including ovarian hyperstimulation syndrome (OHSS)], patient burden and dropout rates [8]. Milder stimulation also intends to obtain better quality oocytes from the cohort of follicles sensitive to exogenous FSH, with the objective that in vivo

T.C. van Tilborg, M.D. • F.J.M. Broekmans, M.D. • H.L. Torrance, M.S. • B.C. Fauser, M.D., Ph.D. (✉)
Department of Reproductive Medicine and Gynaecology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands
e-mail: b.c.fauser@umcutrecht.nl

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_10,

selection will enable more efficient in in vitro identification of the embryos with the best implantation potential.

Despite all these developments, the implantation rate per embryo transferred is still disappointing with a maximum implantation rate of approximately 30 % [9]. This low efficiency seems in a large part due to embryo quality per se. However, endometrium receptivity may also contribute, as evidence exists that secretory endometrium development is often disrupted after COS in comparison to a natural cycle [10]. Improved embryo quality may be achieved through increasing the quality of the retrieved oocytes. This means the focus of ovarian stimulation should move away from quantity and become directed at quality. With the current limitations in effective embryo selection, even for high-technology chromosome assessment on blastocysts [11, 12], aiming for a number of oocytes that represents the optimal range for the chance of obtaining a live birth seems a best way to go.

10.2 Ovarian Physiology

Ovarian function in the female adult is both autonomous and directed by the hypothalamic–pituitary axis. The continuous recruitment of primordial follicles to develop towards the antral stages and the elimination of the vast majority of these developing follicles along the way are fully under control of local factors including bone morphogenetic protein-15 (BMP-15) and anti-Müllerian hormone (AMH) [13, 14]. It is from the small antral stage of follicular development onwards, that pituitary gonadotropin hormones dictate the cyclic follicle recruitment that enables the occurrence of the menstrual cycle (Fig. 10.1) [15].

The attainment of FSH sensitivity in antral follicles from the 1–2 mm stages onwards results from increasing numbers of membrane receptors on the granulosa cells. Up to a follicle diameter of 5 mm only minute amounts of gonadotropins are sufficient for follicle development [16, 17]. For the development into a dominant pre-ovulatory follicle, exposure to higher levels of FSH is necessary. During that development, which takes about 2 weeks, the follicle will increase in size from 5 to about 20–25 mm just before ovulation [18].

Although the number of follicles that are present in the ovary in the small antral stage (2–5 mm) can amount to 25, only one follicle is selected to become the dominant follicle that will subsequently ovulate. The mechanism underlying this single dominant follicle selection has become known as the threshold/window concept. Corpus

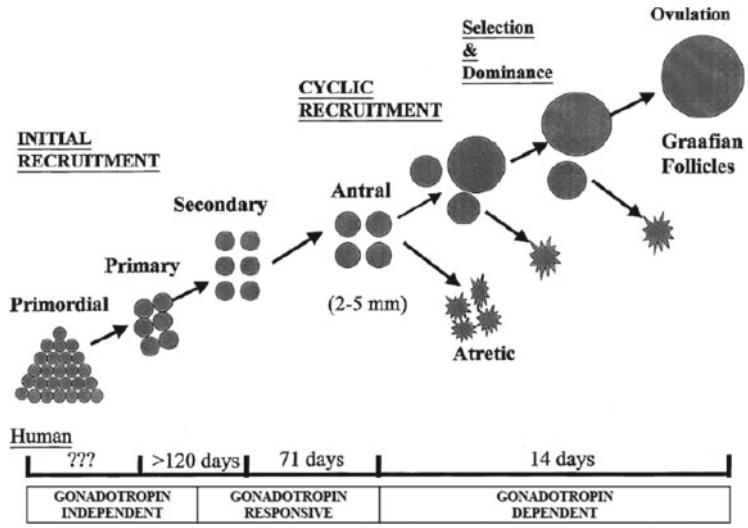

Fig. 10.1 Schematic representation of life history of ovarian follicles: endowment and maintenance, initial recruitment, ovulation and exhaustion (Broekmans et al. [19], permission requested) [15]

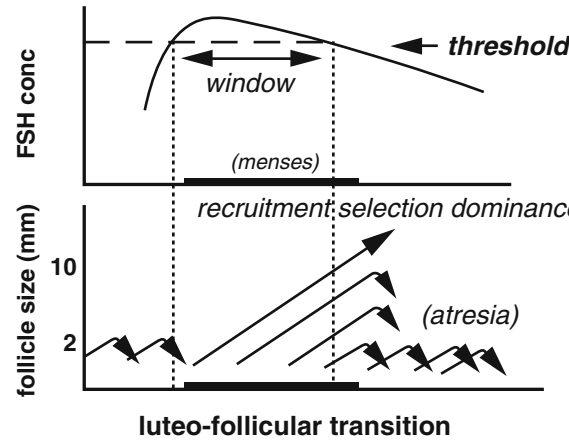

Fig. 10.2 The intercycle rise in FSH concentrations exceeds the threshold for recruitment of a cohort of follicles for further development. The number of follicles recruited is determined by the time ("window") for which the serum FSH is above the threshold at which recruitment occurs. *FSH* follicle stimulating hormone (Macklon and Fauser [20], permission requested) [76]

luteum demise at the end of the previous menstrual cycle and the resulting decrease in oestradiol (E2) and inhibin A levels [21, 22] will cause FSH levels to rise [23]. By surpassing a threshold [23–25], the cohort of FSH-sensitive antral follicles will start to grow and thereby is initially rescued from atresia. Rising FSH levels will however soon become suppressed by negative feedback from E2 [26] and inhibin B [27] produced by the cohort of developing antral follicles. Decreasing FSH levels provide the occurrence of a window or time period in which the individual follicle FSH threshold can be surpassed [15, 28]. The length of the time window and the hierarchy of FSH sensitivity of the various follicles in the cohort will determine the number of follicles that are allowed to begin pre-ovulatory development (dominant follicle growth). In normal physiology, only one or sometimes two follicles will develop and ovulate. Increasing the FSH window by exogenous manipulation will therefore allow the development of several or all of the available antral follicles (Fig. 10.2) [29, 30].

10.3 Mechanism of Controlled Ovarian Stimulation

During COS, normal ovarian physiology is disrupted by follicular phase exogenous gonadotropin administration. By administering compounds that increase the FSH serum concentration, the period in which the FSH threshold is exceeded will become extended [31]. Although differences may exist in FSH sensitivity within the cohort of follicles, overriding the endogenous FSH pattern by for instance exogenous FSH administration will easily lead to the growth of several follicles into dominance [25, 32].

10.3.1 Ovarian Stimulation Agents

The first IVF baby was born after natural cycle IVF [33]. Soon after this ground breaking event, IVF was carried out with ovarian stimulation by CC and/or gonadotropin co-treatment [5]. The availability of more oocytes and embryos for transfer rapidly resulted in higher pregnancy rates after IVF treatment [34, 35]. In current clinical practice, gonadotropins administered in doses ranging from 100 to 600 IU/day combined with GnRH analogue co-treatment are the principal regimen for COS in IVF [5, 6, 36, 37]. This combination is used because exogenous ovarian stimulation by gonadotropins causes a premature luteinizing hormone (LH) surge in 20–25 % of the stimulation cycles [5], leading to high cancellation rates, untimely ovum pickup planning and lower pregnancy rates. This problem is largely solved by GnRH analogue co-treatment [38]. We will discuss two types of GnRH analogues (GnRH agonists and GnRH antagonists) below.

10.3.2 GnRH Analogues

The GnRH decapeptide is intermittently secreted into the portal circulation by the hypothalamus, thereby stimulating pituitary secretion of LH and FSH [39]. Repeated administration of GnRH agonists leads to desensitisation of the pituitary GnRH receptors, resulting in falling LH and FSH levels [40] after an initial stimulation phase ("flare-up") [41]. Pituitary down-regulation starting in the cycle prior to starting COS has been standard practice since 1988 and is known as the "long protocol" [41]. Although highly successful, this protocol also has undesirable side effects, mainly related to oestrogen deprivation and length of treatment [42, 43].

In 2001, two third-generation GnRH analogues (ganirelix and cetrorelix) were registered for use in IVF treatment. Administration of these GnRH antagonists leads to a direct suppression of the pituitary function, along with a rapid recovery after cessation, thereby making this protocol appropriate for starting the GnRH analogue administration during COS. Furthermore, the use of ovarian stimulation during the normal menstrual cycle may enable more IVF cycles to be carried out in a given time period [44]. The reported disadvantages of this protocol include less flexibility regarding cycle planning, and a trend towards lower pregnancy chances per cycle [45, 46].

The long GnRH agonist protocol, in which agonist administration is started on cycle day 21, will prevent the luteo-follicular rise in FSH levels that dictates the antral follicle cohort behaviour towards monofollicular growth. Subsequent exposure to exogenous FSH will lead to a synchronised development of as many follicles as present at the start of stimulation. In contrast, the GnRH antagonist protocol does not suppress endogenous FSH levels during the transition to the follicular phase and normal antral follicle cohort behaviour will be maintained. After exogenous FSH administration is initiated, the FSH window will be extended and additional follicles will be stimulated to grow but in a more asynchronised fashion and leaving some of the follicles unresponsive [47].

10.3.3 FSH Dose Response Relation

From studies on FSH serum levels during ovarian hyperstimulation in conventional protocols, it has been suggested that differences in ovarian response may at least in part be explained by differences in FSH serum levels [48]. However, when using stimulation dosages of 225 IU of hMG, threshold FSH serum levels are highly surpassed, irrespective of response magnitude (FSH serum levels ≥20 IU/l) [48]. This indicates that maximal stimulation may have been applied in all response types, implicating that other factors, such as the available number of FSH-sensitive follicles, play an important role. Indeed, studies on the relationship between baseline FSH, as indicator of antral follicle number, and response to standard doses of exogenous FSH have indicated a dominant role for cohort size [49]. In addition, small increments in exposure to FSH may produce some degree of a dose–response relation, but use of dosages of over 150–225 IU of FSH daily will hardly elicit higher numbers of oocytes [36]. Sterrenburg et al. stated in a systematic review that the optimal daily rFSH dose is 150 IU in presumed normal responders younger than 39 years. This dose resulted in a slightly more modest oocyte yield, but an equal pregnancy rate compared to doses of 225–250 IU/day. Additionally, the number of frozen embryos available for transfer did not improve from dosages over 150 IU/day, suggesting that the cumulative pregnancy rate may not improve by using a higher rFSH dose.

All this means that the number of antral follicles that will respond to ovarian hyperstimulation mainly depends on what the ovaries have in stock at the time of initiation of the stimulation. This number may vary in individuals from cycle to cycle and possibly even from day to day [50]. It may explain why patients with a poor response may seemingly respond better to higher FSH dosages in a subsequent treatment cycle, while those who do not will easily remain "unnoticed". This is especially true as studies proving a benefit from using higher dosages in predicted or actual poor responders are virtually lacking [51–53] or urgently need confirmation [54].

10.4 Types of Ovarian Response

In the available literature, no universally accepted definition of normal, poor or excessive response to ovarian stimulation is used, making it difficult to compare treatment outcomes [55, 56].

10.4.1 Poor Response

The prevalence of a poor response is reported to vary between 5.6 % and 35.1 % [57]. This large variation may stem from differences in the definition of poor response. Recently, the following definition for poor ovarian response (POR) in clinical research has been stated by the European Society of Human Reproduction and Embryology [58]: at least two of the following three features must be present (1) advanced maternal age (≥40 years) or any other risk factor for POR; (2) a previous POR (≤3 oocytes with a conventional stimulation protocol) and (3) an abnormal ovarian reserve test. It is of note that a poor responder can be identified without being stimulated by gonadotropins. It is preferable to refer to these patients as *predicted* poor ovarian responders.

In general, the prevalence of a POR increases with age [58], although even young women can respond poorly to COS [59]. Overall, poor responders have a lower pregnancy chance in comparison to normal responders, with female age and the exact number of oocytes obtained serving as modifiers of this reduced chance [57]. POR is mainly caused by a diminished ovarian reserve, with suboptimal exposure to gonadotropins or the presence of low-sensitive FSH receptor subtypes being more rare explanations. Also, as explained in the previous paragraph, the type of stimulation regime used must be taken into account when judging the type of ovarian response.

10.4.2 Excessive Response

In most literature an excessive response is stated as the retrieval of more than 14–21 oocytes [60]; nevertheless, a uniform definition is lacking. Patients with such a high response to ovarian stimulation have long been viewed as the optimal outcome group. However, from older literature [61], but recently reinforced from large datasets, an excessive response will not automatically lead to optimal pregnancy prospects. Yields over 15–20 oocytes are even associated with reduced live birth rates [62, 63]. These findings are consistent with the assumption that only the most sensitive follicles in stock are likely to yield high-quality oocytes leading to high-quality embryos. The additional oocytes retrieved after maximal stimulation are unlikely to be of such quality that they will lead to implantation. In line with this, increased proportions of low-quality oocyte have been reported in excessive responders [64, 65]. Further explanations for reduced live birth rate in excessive responders are that the excessive E2 levels may directly influence oocyte quality [63, 66, 67] or lead to a reduction in endometrium receptivity [63, 66, 68–70].

Importantly, the high responder patient may experience more discomfort and higher risks for developing OHSS. Up to 30 % of IVF cycles in excessive responders are accompanied by mild-to-moderate OHSS. In 3–8 a severe form of OHSS will develop [71].

10.4.3 Normal Response

If we take into account the definitions of poor response and excessive response stated above, a response leading to 4–21 oocytes may be classified as normal. However, inconsistency in this definition remains. The prevalence of a normal ovarian response defined as ≥4 or ≤15 oocytes in over 2,400 cycles in a fertility clinic in Denmark has been reported to be 70 % [54]. The desired response and the number of oocytes retrieved in the context of the optimal balance between costs, burden of treatment and pregnancy rates remain to be established.

10.5 How to Predict Ovarian Response

From our knowledge on the variability in the timing of reproductive decline, the loose connection between a woman's chronological age and her

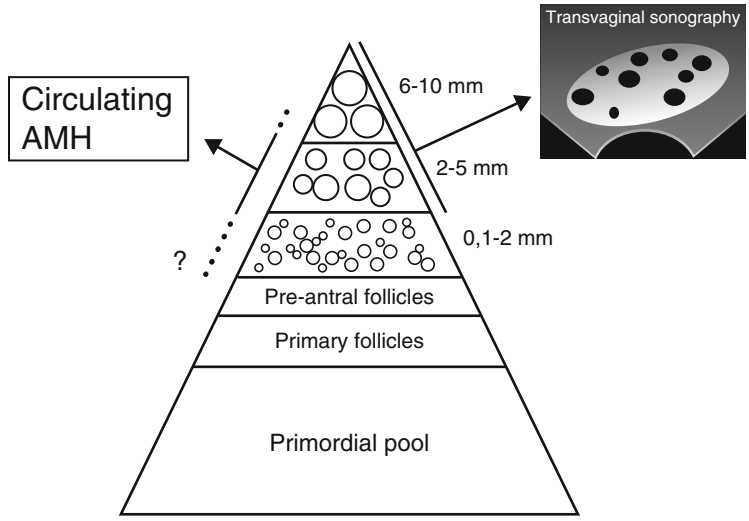

Fig. 10.3 Serum AMH is produced from the cohort of ultrasonically visible antral follicles up to 7 mm. Moreover, follicles below the sensitivity limits of ultrasonography may also contribute to serum levels. This is based on the observation that serum AMH levels do not fall to zero when FSH-sensitive antral follicles (2–5 mm) are stimulated into larger, dominant follicles during ovarian hyperstimulation for IVF and interrupt their AMH production. The *black line* and *dots* represent the stages of antral follicles that contribute to serum AMH. The *grey line* represents the ultrasonically visible antral follicles. *AMH* anti-Müllerian hormone, *FSH* follicle stimulating hormone, *IVF* in vitro fertilisation (Broer et al. COOG [85], permission requested) [21]

reproductive capacity has become apparent [72]. Young women with advanced ovarian ageing may produce a poor response to stimulation and have pregnancy prospects that are below the norm for their age. In contrast, older women with delayed ageing will still produce many oocytes and show quite adequate fertility. Assessment of the biological ovarian age would be necessary to provide information regarding the status of each woman's ovarian reserve and consequently may lead to individualised patient counselling and treatment. To this purpose, ovarian reserve assessment tests (ORTs) have been studied extensively over the last decades. An ideal ORT must reliably measure the quantity of the primordial follicle pool and the overall quality of the oocytes. Unfortunately, it is currently impossible to establish these desired parameters directly [13, 73]. In current practice, ORTs provide an impression of the cohort of recruited antral follicles at the start of each menstrual cycle [13, 15]. The predictive values of ORTs for ovarian response after COS have been analysed on single performance but also in a combination with other tests. Currently, AMH and the Antral Follicle Count (AFC) must be considered as the most practical, reliable and accurate markers of the ovarian reserve and will therefore be discussed in detail below [74–76] (Fig. 10.3).

10.5.1 Anti-Müllerian Hormone

AMH is a member of the transforming growth factor superfamily [77] and is produced in the ovaries, specifically by the granulosa cells in follicles up to 8 mm in diameter [78]. In larger antral follicles (6–8 mm in diameter), AMH expression declines and it becomes undetectable in the pre-ovulatory stage [78, 79]. AMH production in granulosa cells is independent of FSH exposure and it is considered to exert its biological actions mainly in the initial and cyclic recruitment stages of folliculogenesis [13, 80].

It is generally assumed that serum AMH is correlated to a steady pool of small antral follicles, most of which are visible at transvaginal ultrasound [50]. Serum AMH levels are considered

the earliest endocrine marker of the ovarian ageing process [87, 82] and will become undetectable a few years before menopause [83, 84]. A single measurement currently has shown to be highly correlated with the ovarian response to COS, making the test useful for prior response prediction [60, 85].

There is much debate regarding AMH serum cut-off levels for clinical practice. As stated in the ESHRE consensus of defining POR, the best AMH cut-off levels for predicting a poor response range from 0.5 to 1.1 ng/ml [58]. On the other end of the spectrum, it seems that basal AMH levels >3.5 ng/ml are good predictors of hyperresponse and OHSS [86, 87]. Still, there is debate ongoing regarding the reliability of currently available assay systems and improvement of the assay is urgently needed [88–91].

10.5.2 Antral Follicle Count

The AFC is assessed by transvaginal ultrasound examination, counting all the small follicles (2–5 or 2–10 mm in diameter) during the early follicular phase. It is the most commonly used ultrasound marker of ovarian reserve, due to its ease of measurement and reliability [92, 93]. There is considerable variation in AFC between women, whereby age alone mostly explains the decline over time [94]. Besides the intersubject variability in AFC, van Disseldorp et al. [50] reported a higher intra- and intercycle variability within one woman for the AFC compared to AMH. Despite this finding, a low (AFC < 5–7) [58] or high AFC (>15) [60] has been associated with an increased risk for poor or hyperresponse to COS, respectively. Overall, the AFC therefore seems to be a reliable marker for predicting the ovarian response to COS.

It is difficult to compare the available individual studies on the predictive values of ORTs due to the large heterogeneity in the reported studies. Broer et al. [95] recently published an individual patient data meta-analysis, which estimates the added value of ORTs in women undergoing IVF. This study showed that both AMH and AFC had a high accuracy in predicting poor response (AUC 0.78 and 0.76, respectively). A multivariable prediction model consisting of AMH, AFC and age did not lead to a significantly better prediction model than AMH or AFC alone (Fig. 10.4). Also, AMH and AFC have an equal level of accuracy in the prediction of excessive ovarian response without statistical significant differences between those tests [60] (Fig. 10.5).

As stated before, the ovarian decline varies within age groups. Therefore, it can be of added value to identify the ovarian reserve and establish the chance of an ongoing pregnancy and a live birth within specific age groups. AMH and AFC are the most promising markers for predicting ovarian response, and these ORTs can be integrated in individualised COS protocols in order to achieve an appropriate response.

10.6 How to Predict Ongoing Pregnancy

As mentioned above, the definition of IVF success should be shifted from single cycle outcome towards a healthy singleton live birth achieved from a 1-year treatment horizon. It is therefore important to evaluate the predictive value of ORTs for live birth in *consecutive* treatment cycles. Van Disseldorp et al. [96] showed that selection of women with a favourable ovarian reserve status in the female age group 41–43 years led to disappointing results in terms of cumulative live birth rates after IVF. With respect to the outcome ongoing pregnancy, of which available evidence is also scarce, one study reported the predictive value of ORTs in consecutive treatment cycles and reported that age was the only predictive factor [97]. Broer et al. [95] recently confirmed that age is the strongest predictor for ongoing pregnancy (AUC 0.57). In their individual patient data meta-analysis, no single or combined ORT added significant predictive power to the parameter age. These findings confirm results of previous research [74, 76, 98]. In contrast to these studies, La Marca et al. [99] constructed a formula containing both AMH and age, which can be used to calculate the probability of a live birth following the first IVF

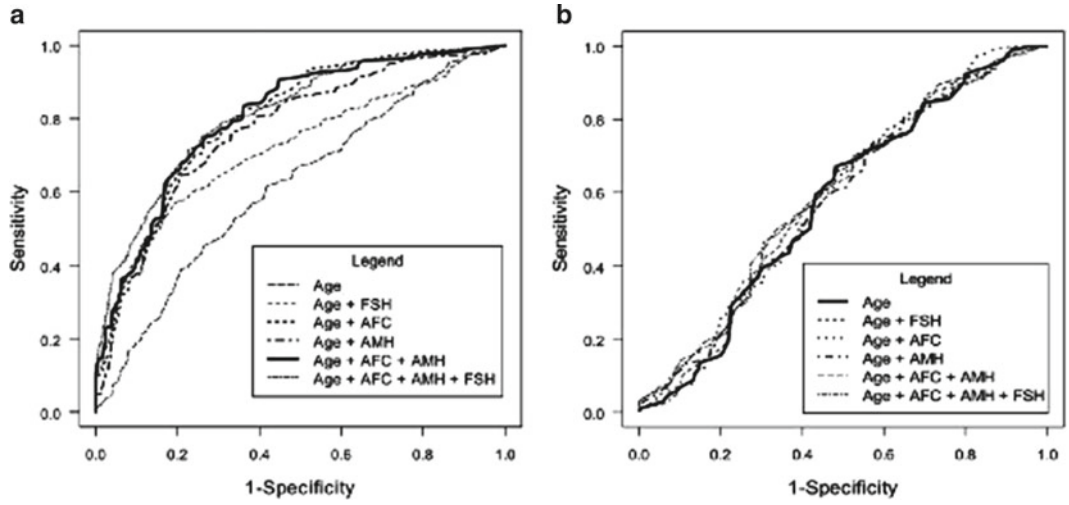

Fig. 10.4 ROC curves of age and ORT(s) in the prediction of poor response and ongoing pregnancy. (**a**) Poor response prediction based on age and ORT. The ROC curves of age or age combined with a single or multiple ORT(s) are depicted. The ROC curves for "Age+AMH", "Age+AMH+AFC" and "Age+AMH+AFC+FSH" run towards the *upper left* corner, indicating a good capacity to discriminate between normal and poor responders at certain cut-off levels. (**b**) Ongoing pregnancy prediction based on age and ORT(s). The ROC curves age or age combined with one or more ORTs run almost parallel to or even cross the X=Y line, indicating that the tests are useless for pregnancy prediction. *ROC* receiver operating characteristic, *ORTs* ovarian reserve assessment tests, *AMH* anti-Müllerian hormone, *AFC* antral follicle count, *FSH* follicle stimulating hormone (Broer et al. [95], permission requested) [23]

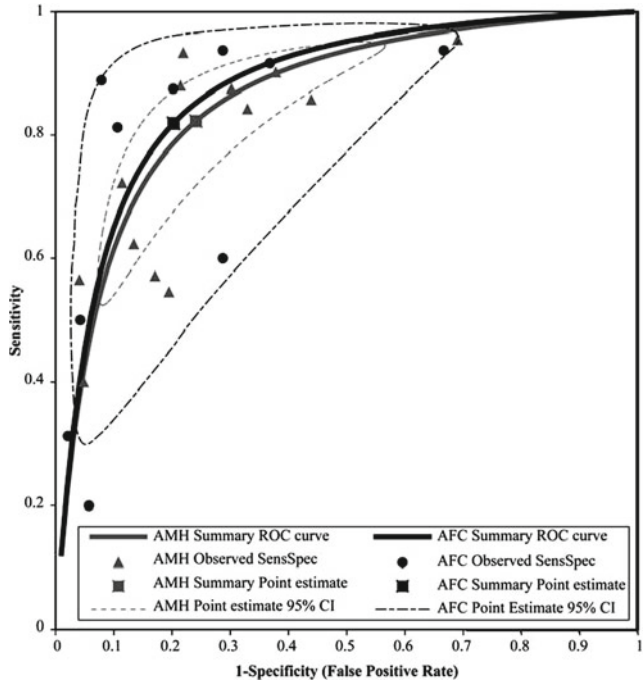

Fig. 10.5 ROC curves of AMH and AFC in the prediction of an excessive response. Note: regardless of the number of cut-offs mentioned per study, only one cut-off was taken into analysis. For the observed values of sensitivity-specificity points, all cut-offs are displayed. *ROC* receiver operating characteristic, *AMH* anti-Müllerian hormone, *AFC* antral follicle count (Broer et al. [60], permission requested) [17]

treatment cycle. They concluded that moderate distinction (ROC_{auc} 0.66) at all female ages can be made between couples with a good or poor prognosis. However, confirmation and validation of this model needs to be awaited.

Currently, clear cut-off values for clinical practice in order to predict ongoing pregnancy or live birth are not available. Pregnancies in IVF patients may even occur in women with undetectable AMH levels.

10.7 How to Influence Ovarian Response and Ongoing Pregnancy Rates

Although the prediction of ovarian response categories using AMH and/or the AFC is accurate, the clinical value of this finding depends on the consequences these tests have for patient management. Both the questions of which management options should be chosen based on the test result, as well as to what extent cost-effectiveness will increase by this policy need to be evaluated. Clinical implications of abnormal test results could vary from counselling the patient regarding the expected response to ovarian hyperstimulation to changing patient management by for example FSH dose adjustments or the use of a specific stimulation protocol.

To date, studies addressing individualised regimens based on ovarian reserve testing have provided contradictory results [51, 53, 54, 100, 101]. In a randomised study, doubling the starting dose of gonadotropins from 150 to 300 IU/day in predicted poor responders (defined as an AFC < 5) did not lead to improvement of the response to stimulation or pregnancy prospects [53]. In a comparable, but pseudo-randomised design, it was demonstrated that increasing the starting dose of FSH stimulation in potential poor responders based on low AMH values did not alter response or pregnancy rates [100]. Also, the effect of two high dose FSH treatment arms (300 versus 400 IU daily) in predicted poor responders based on basal FSH levels was studied. Despite a sufficient ovarian response in both dosage arms, the outcome at all stages of the IVF treatment was still equally poor and clearly poorer than in women with normal FSH levels (Fig. 10.6) [51]. In remarkable contrast to these three studies, an individualised starting dose based on a response predicting algorithm did in fact narrow the distribution of ovarian response and did reduce the incidence of patients with a poor or excessive response [54]. These results were confirmed by a study demonstrating that an individual dose resulted in fewer cancellations for excessive response [101, 102]. Popovic-Todorovic et al. [54] also showed that individualised dosing may lead to improved pregnancy rates, a finding that still needs to be confirmed in other studies.

In addition to these randomised comparative studies, a few non-randomised trials have been carried out in order to demonstrate the improved efficacy or cost-efficacy of individualised patient management. Yates et al. [103] conducted a retrospective comparison study with a historical control group on first IVF cycles in women with an AFC ≥ 8 and AMH > 2.2 pmol/l. Conventional stimulation based on basal FSH measurements was compared to AMH based tailored protocols. A significant increase in embryo transfer rate, pregnancy rate per cycle started, and live birth rate, and a lower incidence of OHSS and lower costs per patient in favour of AMH-tailored protocols was demonstrated. Additionally, Nelson et al. [104] conducted a prospective centre comparison study in which 538 patients undergoing their first IVF treatment were classified based on their AMH serum levels. They reported that the use of a GnRH antagonist led to a significant reduction in the rate of excessive response, defined as >21 oocytes yielded, compared to a GnRH agonist scheme in predicted hyperresponders (AMH ≥ 15 pmol/l). The need for complete cryopreservation was clearly reduced, as was the cancellation rate, with also a significant increase in clinical pregnancy rate per started cycle [21/34 (61.7 %) and 47/148 (31.8 %), respectively]. It appears that the GnRH antagonist protocol indeed may have a better safety profile, evidenced by a significant reduction in the chance of developing OHSS, related to a modest reduction in ovarian response [46, 105].

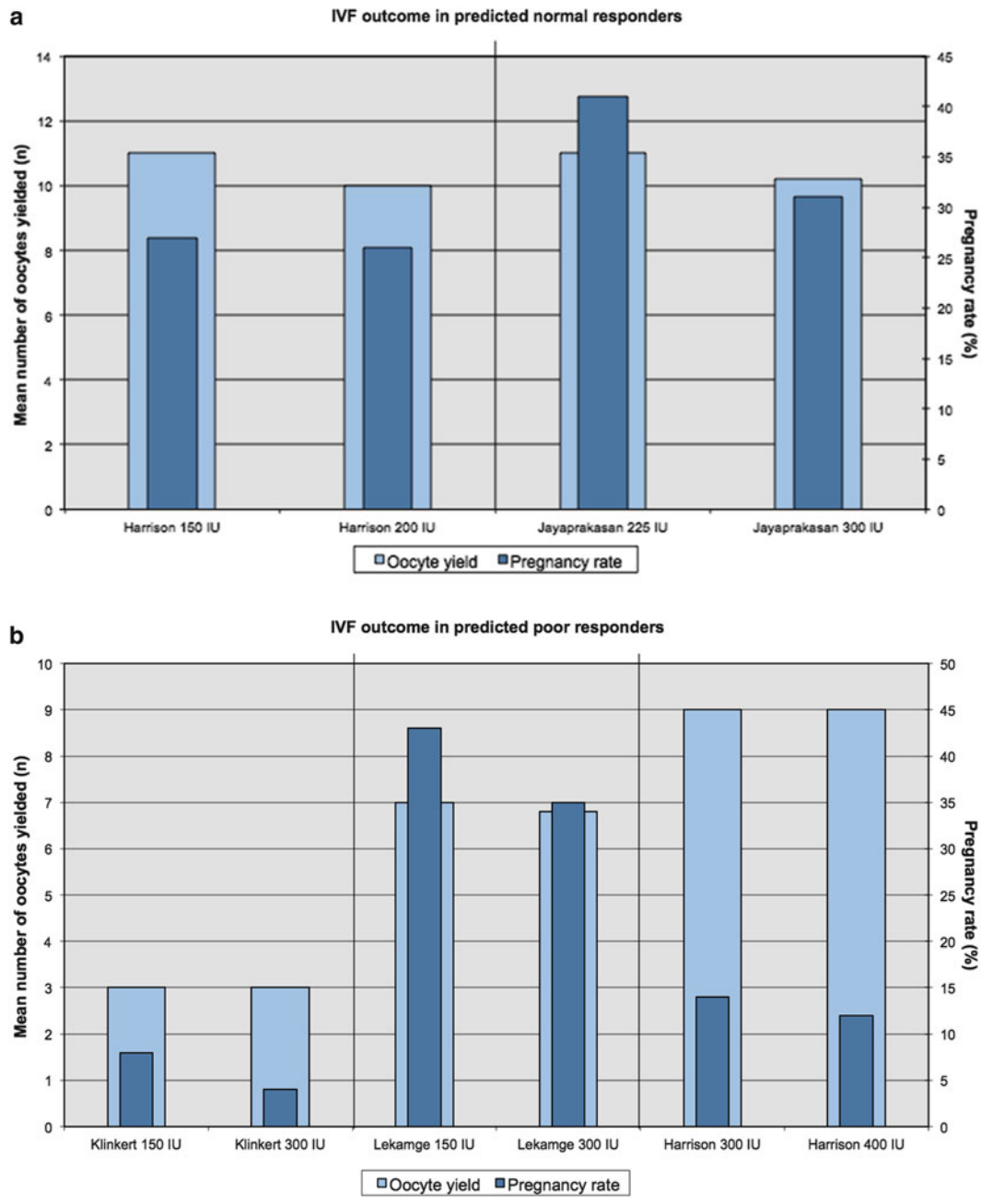

Fig. 10.6 IVF outcome according to FSH dose from RCTs. The IVF outcome is represented by the mean number of oocytes yielded and pregnancy rate per cycle, in predicted normal and poor responders. Data were extracted from the following articles: Harrison et al. [51], Jayaprakasan et al. [52], Lekamge et al. [100] and Klinkert et al. [53]. (**a**) IVF outcome in predicted normal responders. No significant differences on oocyte yield and clinical pregnancy rate (Harrison et al. [51]) or live birth rate (Jayaprakasan et al. [52]) per started cycle was found between the different FSH doses. (**b**) IVF outcome in predicted poor responders. No significant differences on oocyte yield and clinical pregnancy rate (Harrison et al. [51]) or ongoing pregnancy rate (Lekamge et al. [100], Klinkert et al. [53]) per started cycle were found between the different FSH doses. *RCTs* randomised controlled trials, *IVF* in vitro fertilisation, *FSH* follicle stimulating hormone

On the other hand, in patients older than 40 years, in which a diminished ovarian reserve can be expected, it seems that the long agonist protocol performed better than the GnRH antagonist protocol [106]. These studies demonstrate the possible power of individualised management, by means of FSH dose adjustment and/or GnRH agonist or antagonist administration, based on ovarian reserve testing (Fig. 10.7), but need confirmation in well-designed randomised controlled trials.

Currently, the OPTIMIST trial (OPTIMisation of cost-effectiveness through Individualised FSH Stimulation dosages of IVF Treatment: a randomised trial, registration nr: NTR2657) and the CONSORT study (CONSistency in r-FSH starting dOses for individualised tReatmenT, registration nr: NCT00829244) are being performed or have been finalised and will help to answer the questions stated above by determining whether individualised dosing based on ORTs prior to IVF treatment have indeed clinical value.

Next to adjustments in dosage of FSH or stimulation regime applied, other adjunctive therapies have been studied specifically focusing on improving a poor ovarian response to COS and subsequently pregnancy rates. These therapies include growth hormone (GH) supplements, androgen supplements and recombinant LH (rLH) and are mainly studied in cases with a first cycle poor response. The underlying hypothesis for adding GH in order to improve pregnancy rates in poor responders is that GH plays an important role in ovarian steroidogenesis and follicular development [107]. A Cochrane review has shown that GH co-treatment may in fact increase pregnancy rates

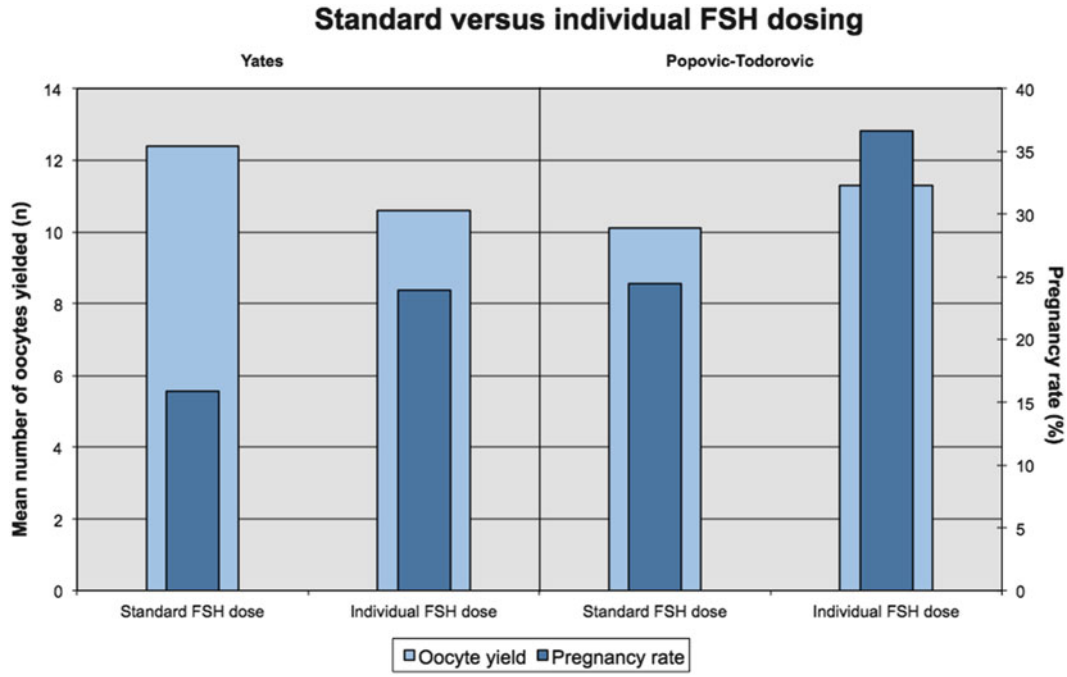

Fig. 10.7 IVF outcome in standard versus individualised FSH dosing protocols. Yates et al. [103] used an AMH-tailored approach, in a non-randomised historical control group design. Popovic-Todorovic et al. [54] used an algorithm based on AFC, total ovarian volume, total Doppler score, age and smoking habits in a RCT design. The IVF outcome is represented by the mean number of oocytes yielded and ongoing pregnancy rate (Popovic-Todorovic et al. [54]) or live birth rate per cycle (Yates et al. [103]). A significant difference in favour of using an individualised approach was found in both studies with respect to the pregnancy or live birth rate. A significant difference with respect to the oocyte yield was only found in Yates et al. [103]. *IVF* in vitro fertilisation, *FSH* follicle stimulating hormone, *AMH* anti-Müllerian hormone, *AFC* antral follicle count, *RCT* randomised controlled trial

in (predicted) poor responders [108]. However, heterogeneity in POR definition and lack of available evidence resulting in wide confidence intervals may limit the implications of these findings. A recently published reassessment of three meta-analyses also showed that GH co-treatment in different POR subgroups is promising; however, good quality evidence is still lacking [109].

The supplementation of androgens for predicted POR relates to the underlying theory that intra-ovarian androgens promote survival and later FSH sensitivity of growing follicles [110, 111] and therefore may increase the number of available antral follicles to be stimulated. The role of various interventions including pre-treatment with transdermal testosterone or dehydroepiandrosterone (DHEA), and addition of aromatase inhibitors, rLH or recombinant hCG during COS in poor responders has recently been evaluated in a systematic review and meta-analysis [112, 113]. Significant differences in clinical pregnancy and live birth rate were found with transdermal testosterone pre-treatment compared to controls [114, 115]. Neither adjuvant therapy by DHEA, rLH or recombinant administration nor the use of aromatase inhibitors resulted in altered clinical pregnancy rates [112, 113, 116, 121]. In line with this, a Cochrane review [122] on LH supplementation shows no evidence for statistical differences in pregnancy rates. Only one study provided data on live birth and rLH addition and [119] reported a significantly increased live birth rate in women who received rLH when compared to controls. Sunkura et al. [113] also recently published a meta-analysis on androgen supplements in poor responders. No significant differences were found for the outcome clinical pregnancy rate by meta-analysis of five RCTs [115, 116, 121, 123, 124] and four non-randomised controlled studies [125–128]. However, a significantly higher clinical pregnancy rate was reported in the study groups that used either testosterone patches or DHEA compared to controls. This finding does not correspond with the previously discussed meta-analysis, which may be due to the inclusion of non-randomised controlled trials.

It is noteworthy that all these trials have several limitations including limited number of patients per study, the absence of a standard POR definition, heterogeneity in dosing, initiation and duration of stimulation and variation in GnRH analogue protocols. Furthermore, effects independent of age were not analysed making it unclear whether the favourable outcomes will apply to any poor responder patient. Currently, transdermal testosterone pre-treatment and GH supplements seem to be of added value in poor responders although this conclusion is based on limited evidence. Further properly designed RCTs are urgently needed to accurately evaluate the added value of androgen and GH supplements in poor responders.

10.8 Conclusion

Patient-tailored approach in assisted reproductive technology (ART) is still under construction. Current available data hold many promises for the overall improvement of IVF programs by individualised choices of the stimulation regimes. AMH and AFC are the most reliable markers for predicting ovarian response to COS and are the basis of large randomised controlled trials from which very soon data will start to emerge. Until that time, it may be emphasised that in predicted or observed poor responders, the usage of high FSH dosages for stimulation, or adjuncts like androgens, may not be justified, while dose reduction or stimulation scheme changes in anticipated high or excessive responders may yield the best gains in terms of success rates and costs.

References

1. Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril. 2009;92(5):1520–4.
2. Gnoth C, Godehardt D, Godehardt E, et al. Time to pregnancy: results of the German prospective study and impact on the management of infertility. Hum Reprod. 2003;18(9):1959–66.

3. Hull MG, Glazener CM, Kelly NJ, et al. Population study of causes, treatment, and outcome of infertility. Br Med J (Clin Res Ed). 1985;291(6510):1693–7.
4. Fauser BC, Mannaerts BM, Devroey P, et al. Advances in recombinant DNA technology: corifollitropin alfa, a hybrid molecule with sustained follicle-stimulating activity and reduced injection frequency. Hum Reprod Update. 2009;15(3):309–21.
5. Macklon NS, Stouffer RL, Giudice LC, et al. The science behind 25 years of ovarian stimulation for in vitro fertilization. Endocr Rev. 2006;27(2):170–207.
6. Nargund G, Fauser BC, Macklon NS, et al. The ISMAAR proposal on terminology for ovarian stimulation for IVF. Hum Reprod. 2007;22(11):2801–4.
7. van Hooff MH, Alberda AT, Huisman GJ, et al. Doubling the human menopausal gonadotrophin dose in the course of an in-vitro fertilization treatment cycle in low responders: a randomized study. Hum Reprod. 1993;8(3):369–73.
8. Heijnen EM, Eijkemans MJ, De Klerk C, et al. A mild treatment strategy for in-vitro fertilisation: a randomised non-inferiority trial. Lancet. 2007; 369(9563):743–9.
9. de Mouzon J, Goossens V, Bhattacharya S, et al. Assisted reproductive technology in Europe, 2006: results generated from European registers by ESHRE. Hum Reprod. 2010;25(8):1851–62.
10. Paulson RJ, Sauer MV, Lobo RA. Embryo implantation after human in vitro fertilization: importance of endometrial receptivity. Fertil Steril. 1990;53(5): 870–4.
11. Kroener L, Ambartsumyan G, Briton-Jones C, et al. The effect of timing of embryonic progression on chromosomal abnormality. Fertil Steril. 2012;98(4): 876–80.
12. Wells D. Embryo aneuploidy and the role of morphological and genetic screening. Reprod Biomed Online. 2010;21(3):274–7.
13. McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. Endocr Rev. 2000;21(2):200–14.
14. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr Rev. 1996;17(2):121–55.
15. Fauser BC, Van Heusden AM. Manipulation of human ovarian function: physiological concepts and clinical consequences. Endocr Rev. 1997;18(1): 71–106.
16. Govan AD, Black WP. Ovarian morphology in oligomenorrhea. Eur J Obstet Gynecol Reprod Biol. 1975;5(6):317–25.
17. Hillier SG. Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. Hum Reprod. 1994;9(2):188–91.
18. Pache TD, Wladimiroff JW, de Jong FH, et al. Growth patterns of nondominant ovarian follicles during the normal menstrual cycle. Fertil Steril. 1990;54(4):638–42.
19. Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: clinical mechanisms and clinical consequences. Endocr Rev. 2009;30(5):465–93.
20. Macklon NS, Fauser BC. Follicle development during the normal menstrual cycle. Maturitas. 1998; 30(2):181–8.
21. le Nestour E, Marraoui J, Lahlou N, et al. Role of estradiol in the rise in follicle-stimulating hormone levels during the luteal-follicular transition. J Clin Endocrinol Metab. 1993;77(2):439–42.
22. Roseff SJ, Bangah ML, Kettel LM, et al. Dynamic changes in circulating inhibin levels during the luteal-follicular transition of the human menstrual cycle. J Clin Endocrinol Metab. 1989;69(5): 1033–9.
23. Hall JE, Schoenfeld DA, Martin KA, et al. Hypothalamic gonadotropin-releasing hormone secretion and follicle-stimulating hormone dynamics during the luteal-follicular transition. J Clin Endocrinol Metab. 1992;74(3):600–7.
24. Brown JB. Pituitary control of ovarian function–concepts derived from gonadotrophin therapy. Aust N Z J Obstet Gynaecol. 1978;18(1):46–54.
25. Schoemaker J, van Weissenbruch MM, Scheele F, et al. The FSH threshold concept in clinical ovulation induction. Baillieres Clin Obstet Gynaecol. 1993;7(2):297–308.
26. Zeleznik AJ, Hutchison JS, Schuler HM. Interference with the gonadotropin-suppressing actions of estradiol in macaques overrides the selection of a single preovulatory follicle. Endocrinology. 1985;117(3): 991–9.
27. Groome NP, Illingworth PJ, O'Brien M, et al. Measurement of dimeric inhibin B throughout the human menstrual cycle. J Clin Endocrinol Metab. 1996;81(4):1401–5.
28. van Santbrink EJ, Hop WC, van Dessel TJ, et al. Decremental follicle-stimulating hormone and dominant follicle development during the normal menstrual cycle. Fertil Steril. 1995;64(1):37–43.
29. Macklon NS, Fauser BC. Follicle-stimulating hormone and advanced follicle development in the human. Arch Med Res. 2001;32(6):595–600.
30. Schipper I, Hop WC, Fauser BC. The follicle-stimulating hormone (FSH) threshold/window concept examined by different interventions with exogenous FSH during the follicular phase of the normal menstrual cycle: duration, rather than magnitude, of FSH increase affects follicle development. J Clin Endocrinol Metab. 1998;83(4):1292–8.
31. Macklon NS, Fauser BC. Gonadotropin therapy for the treatment of anovulation and for ovarian hyperstimulation for IVF. Mol Cell Endocrinol. 2002; 186(2):159–61.
32. Messinis IE, Templeton AA. The importance of follicle-stimulating hormone increase for folliculogenesis. Hum Reprod. 1990;5(2):153–6.
33. Edwards RG, Steptoe PC, Purdy JM. Establishing full-term human pregnancies using cleaving embryos grown in vitro. Br J Obstet Gynaecol. 1980;87(9): 737–56.
34. Healy DL, Okamato S, Morrow L, et al. Contributions of in vitro fertilization to knowledge of the

reproductive endocrinology of the menstrual cycle. Baillieres Clin Endocrinol Metab. 1987;1(1):133–52.
35. Jennings JC, Moreland K, Peterson CM. In vitro fertilisation. A review of drug therapy and clinical management. Drugs. 1996;52(3):313–43.
36. Sterrenburg MD, Veltman-Verhulst SM, Eijkemans MJ, et al. Clinical outcomes in relation to the daily dose of recombinant follicle-stimulating hormone for ovarian stimulation in in vitro fertilization in presumed normal responders younger than 39 years: a meta-analysis. Hum Reprod Update. 2011;17(2): 184–96.
37. [FIVNAT 1996 report. French National Register on In Vitro Fertilization]. Contracept Fertil Sex. 1997; 25(7–8):499–502.
38. Smitz J, Devroey P, Camus M, et al. The luteal phase and early pregnancy after combined GnRH-agonist/ HMG treatment for superovulation in IVF or GIFT. Hum Reprod. 1988;3(5):585–90.
39. Huirne JA, Lambalk CB. Gonadotropin-releasing-hormone-receptor antagonists. Lancet. 2001; 358(9295):1793–803.
40. Conn PM, Crowley Jr WF. Gonadotropin-releasing hormone and its analogs. Annu Rev Med. 1994; 45:391–405.
41. Fauser BC, Devroey P. Why is the clinical acceptance of gonadotropin-releasing hormone antagonist cotreatment during ovarian hyperstimulation for in vitro fertilization so slow? Fertil Steril. 2005; 83(6):1607–11.
42. Goverde AJ, McDonnell J, Vermeiden JP, et al. Intrauterine insemination or in-vitro fertilisation in idiopathic subfertility and male subfertility: a randomised trial and cost-effectiveness analysis. Lancet. 2000;355(9197):13–8.
43. Verberg MF, Eijkemans MJ, Heijnen EM, et al. Why do couples drop-out from IVF treatment? A prospective cohort study. Hum Reprod. 2008;23(9):2050–5.
44. Verberg MF, Macklon NS, Nargund G, et al. Mild ovarian stimulation for IVF. Hum Reprod Update. 2009;15(1):13–29.
45. Tarlatzis BC, Fauser BC, Kolibianakis EM, et al. GnRH antagonists in ovarian stimulation for IVF. Hum Reprod Update. 2006;12(4):333–40.
46. Al-Inany HG, Youssef MA, Aboulghar M, et al. GnRH antagonists are safer than agonists: an update of a Cochrane review. Hum Reprod Update. 2011; 17(4):435.
47. Huirne JA, Homburg R, Lambalk CB. Are GnRH antagonists comparable to agonists for use in IVF? Hum Reprod. 2007;22(11):2805–13.
48. Ben-Rafael Z, Strauss 3rd JF, Mastroianni Jr L, et al. Differences in ovarian stimulation in human menopausal gonadotropin treated woman may be related to follicle-stimulating hormone accumulation. Fertil Steril. 1986;46(4):586–92.
49. Karlsson MO, Wade JR, Loumaye E, et al. A population model for the follicular growth in women treated with follicle stimulating hormone. Clin Pharmacol Ther. 1997;62(6):665–74.
50. van Disseldorp J, Lambalk CB, Kwee J, et al. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. Hum Reprod. 2010;25(1):221–7.
51. Harrison RF, Jacob S, Spillane H, et al. A prospective randomized clinical trial of differing starter doses of recombinant follicle-stimulating hormone (follitropin-beta) for first time in vitro fertilization and intracytoplasmic sperm injection treatment cycles. Fertil Steril. 2001;75(1):23–31.
52. Jayaprakasan K, Hopkisson J, Campbell B, et al. A randomised controlled trial of 300 versus 225 IU recombinant FSH for ovarian stimulation in predicted normal responders by antral follicle count. BJOG. 2010;117(7):853–62.
53. Klinkert ER, Broekmans FJ, Looman CW, et al. Expected poor responders on the basis of an antral follicle count do not benefit from a higher starting dose of gonadotrophins in IVF treatment: a randomized controlled trial. Hum Reprod. 2005;20(3):611–5.
54. Popovic-Todorovic B, Loft A, Bredkjaeer HE, et al. A prospective randomized clinical trial comparing an individual dose of recombinant FSH based on predictive factors versus a 'standard' dose of 150 IU/day in 'standard' patients undergoing IVF/ICSI treatment. Hum Reprod. 2003;18(11):2275–82.
55. Kailasam C, Keay SD, Wilson P, et al. Defining poor ovarian response during IVF cycles, in women aged <40 years, and its relationship with treatment outcome. Hum Reprod. 2004;19(7):1544–7.
56. Tarlatzis BC, Zepiridis L, Grimbizis G, et al. Clinical management of low ovarian response to stimulation for IVF: a systematic review. Hum Reprod Update. 2003;9(1):61–76.
57. Oudendijk JF, Yarde F, Eijkemans MJ, et al. The poor responder in IVF: is the prognosis always poor? A systematic review. Hum Reprod Update. 2012; 18(1):1–11.
58. Ferraretti AP, La Marca A, Fauser BC, et al. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod. 2011;26(7):1616–24.
59. El-Toukhy T, Khalaf Y, Hart R, et al. Young age does not protect against the adverse effects of reduced ovarian reserve–an eight year study. Hum Reprod. 2002;17(6):1519–24.
60. Broer SL, Dolleman M, Opmeer BC, et al. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. Hum Reprod Update. 2011;17(1):46–54.
61. Ben-Rafael Z, Benadiva CA, Ausmanas M, et al. Dose of human menopausal gonadotropin influences the outcome of an in vitro fertilization program. Fertil Steril. 1987;48(6):964–8.
62. Sunkara SK, Rittenberg V, Raine-Fenning N, et al. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. Hum Reprod. 2011;26(7):1768–74.
63. van der Gaast MH, Eijkemans MJ, van der Net JB, et al. Optimum number of oocytes for a successful

first IVF treatment cycle. Reprod Biomed Online. 2006;13(4):476–80.
64. Baart EB, Martini E, Eijkemans MJ, et al. Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. Hum Reprod. 2007; 22(4):980–8.
65. Munne S. Chromosome abnormalities and their relationship to morphology and development of human embryos. Reprod Biomed Online. 2006;12(2): 234–53.
66. Fauser BC, Devroey P, Macklon NS. Multiple birth resulting from ovarian stimulation for subfertility treatment. Lancet. 2005;365(9473):1807–16.
67. Hohmann FP, Macklon NS, Fauser BC. A randomized comparison of two ovarian stimulation protocols with gonadotropin-releasing hormone (GnRH) antagonist cotreatment for in vitro fertilization commencing recombinant follicle-stimulating hormone on cycle day 2 or 5 with the standard long GnRH agonist protocol. J Clin Endocrinol Metab. 2003; 88(1):166–73.
68. Macklon NS, Fauser BC. Impact of ovarian hyperstimulation on the luteal phase. J Reprod Fertil Suppl. 2000;55:101–8.
69. Simon C, Garcia Velasco JJ, Valbuena D, et al. Increasing uterine receptivity by decreasing estradiol levels during the preimplantation period in high responders with the use of a follicle-stimulating hormone step-down regimen. Fertil Steril. 1998;70(2): 234–9.
70. Van Der Gaast MH, Beckers NG, Beier-Hellwig K, et al. Ovarian stimulation for IVF and endometrial receptivity – the missing link. Reprod Biomed Online. 2002;5 Suppl 1(3):36–43.
71. Delvigne A, Rozenberg S. Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS): a review. Hum Reprod Update. 2002;8(6):559–77.
72. te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update. 2002; 8(2):141–54.
73. Gougeon A. Ovarian follicular growth in humans: ovarian ageing and population of growing follicles. Maturitas. 1998;30(2):137–42.
74. Broekmans FJ, Kwee J, Hendriks DJ, et al. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update. 2006;12(6): 685–718.
75. La Marca A, Sighinolfi G, Radi D, et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update. 2010;16(2):113–30.
76. Broer SL, Mol BW, Hendriks D, et al. The role of antimullerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. Fertil Steril. 2009;91(3):705–14.
77. Cate RL, Mattaliano RJ, Hession C, et al. Isolation of the bovine and human genes for Mullerian inhibiting substance and expression of the human gene in animal cells. Cell. 1986;45(5):685–98.
78. Weenen C, Laven JS, Von Bergh AR, et al. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod. 2004;10(2): 77–83.
79. Andersen CY, Schmidt KT, Kristensen SG, et al. Concentrations of AMH and inhibin-B in relation to follicular diameter in normal human small antral follicles. Hum Reprod. 2010;25(5):1282–7.
80. Gruijters MJ, Visser JA, Durlinger AL, et al. Anti-Mullerian hormone and its role in ovarian function. Mol Cell Endocrinol. 2003;211(1–2):85–90.
81. La Marca A, Giulini S, Tirelli A, et al. Anti-Mullerian hormone measurement on any day of the menstrual cycle strongly predicts ovarian response in assisted reproductive technology. Hum Reprod. 2007;22(3): 766–71.
82. de Vet A, Laven JS, de Jong FH, et al. Antimullerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril. 2002;77(2):357–62.
83. Broer SL, Eijkemans MJ, Scheffer GJ, et al. Anti-mullerian hormone predicts menopause: a long-term follow-up study in normoovulatory women. J Clin Endocrinol Metab. 2011;96(8):2532–9.
84. Sowers MR, Eyvazzadeh AD, McConnell D, et al. Anti-mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. J Clin Endocrinol Metab. 2008;93(9):3478–83.
85. Broer SL, Mol B, Dolleman M, et al. The role of anti-Mullerian hormone assessment in assisted reproductive technology outcome. Curr Opin Obstet Gynecol. 2010;22(3):193–201.
86. Lee TH, Liu CH, Huang CC, et al. Impact of female age and male infertility on ovarian reserve markers to predict outcome of assisted reproduction technology cycles. Reprod Biol Endocrinol. 2009;7:100.
87. Nardo LG, Gelbaya TA, Wilkinson H, et al. Circulating basal anti-Mullerian hormone levels as predictor of ovarian response in women undergoing ovarian stimulation for in vitro fertilization. Fertil Steril. 2009;92(5):1586–93.
88. Broekmans FJ, Visser JA, Laven JS, et al. Anti-Mullerian hormone and ovarian dysfunction. Trends Endocrinol Metab. 2008;19(9):340–7.
89. Grynnerup AG, Lindhard A, Sorensen S. The role of anti-Mullerian hormone in female fertility and infertility – an overview. Acta Obstet Gynecol Scand. 2012;91(11):1252–60.
90. Li HW, Ng EH, Wong BP, et al. Correlation between three assay systems for anti-Mullerian hormone (AMH) determination. J Assist Reprod Genet. 2012;29(12):1443–6.
91. Rustamov O, Smith A, Roberts SA, et al. Anti-Mullerian hormone: poor assay reproducibility in a large cohort of subjects suggests sample instability. Hum Reprod. 2012;27(10):3085–91.
92. Broekmans FJ, de Ziegler D, Howles CM, et al. The antral follicle count: practical recommendations for better standardization. Fertil Steril. 2010;94(3): 1044–51.

93. Scheffer GJ, Broekmans FJ, Bancsi LF, et al. Quantitative transvaginal two- and three-dimensional sonography of the ovaries: reproducibility of antral follicle counts. Ultrasound Obstet Gynecol. 2002;20(3):270–5.
94. La Marca A, Spada E, Sighinolfi G, et al. Age-specific nomogram for the decline in antral follicle count throughout the reproductive period. Fertil Steril. 2011;95(2):684–8.
95. Broer SL, van Disseldorp J, Broeze KA, et al. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach. Hum Reprod Update. 2013;19(1):26–36.
96. van Disseldorp J, Eijkemans MJ, Klinkert ER, et al. Cumulative live birth rates following IVF in 41- to 43-year-old women presenting with favourable ovarian reserve characteristics. Reprod Biomed Online. 2007;14(4):455–63.
97. Hendriks DJ, te Velde ER, Looman CW, et al. The role of poor response in the prediction of the cumulative ongoing pregnancy rate in in vitro fertilisation. In: Dynamic and Basal Ovarian Reserve Tests for Outcome Prediction in IVF: Comparisons and Meta-Analyses. Academic Thesis: Utrecht; 2005. p. 162–179.
98. Verhagen TE, Hendriks DJ, Bancsi LF, et al. The accuracy of multivariate models predicting ovarian reserve and pregnancy after in vitro fertilization: a meta-analysis. Hum Reprod Update. 2008;14(2): 95–100.
99. La Marca A, Nelson SM, Sighinolfi G, et al. Anti-Mullerian hormone-based prediction model for a live birth in assisted reproduction. Reprod Biomed Online. 2011;22(4):341–9.
100. Lekamge DN, Lane M, Gilchrist RB, et al. Increased gonadotrophin stimulation does not improve IVF outcomes in patients with predicted poor ovarian reserve. J Assist Reprod Genet. 2008;25(11–12): 515–21.
101. Olivennes F, Howles CM, Borini A, et al. Individualizing FSH dose for assisted reproduction using a novel algorithm: the CONSORT study. Reprod Biomed Online. 2009;18(2):195–204.
102. Gleicher N, Weghofer A, Barad DH. Improvement in diminished ovarian reserve after dehydroepiandrosterone supplementation. Reprod Biomed Online. 2010;21(3):360–5.
103. Yates AP, Rustamov O, Roberts SA, et al. Anti-Mullerian hormone-tailored stimulation protocols improve outcomes whilst reducing adverse effects and costs of IVF. Hum Reprod. 2011;26(9): 2353–62.
104. Nelson SM, Yates RW, Lyall H, et al. Anti-Mullerian hormone-based approach to controlled ovarian stimulation for assisted conception. Hum Reprod. 2009; 24(4):867–75.
105. Al-Inany HG, Youssef MA, Aboulghar M, et al. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Syst Rev. 2011;5:CD001750.
106. Sbracia M, Colabianchi J, Giallonardo A, et al. Cetrorelix protocol versus gonadotropin-releasing hormone analog suppression long protocol for superovulation in intracytoplasmic sperm injection patients older than 40. Fertil Steril. 2009;91(5):1842–7.
107. Kolibianakis EM, Venetis CA, Diedrich K, et al. Addition of growth hormone to gonadotrophins in ovarian stimulation of poor responders treated by in-vitro fertilization: a systematic review and meta-analysis. Hum Reprod Update. 2009;15(6):613–22.
108. Ahmad G, Brown J, Duffy JM, et al. Growth hormone for in vitro fertilization. Cochrane Database Syst Rev. 2009;4:CD000099.
109. de Ziegler D, Streuli I, Meldrum DR, et al. The value of growth hormone supplements in ART for poor ovarian responders. Fertil Steril. 2011;96(5): 1069–76.
110. Hillier SG, De Zwart FA. Evidence that granulosa cell aromatase induction/activation by follicle-stimulating hormone is an androgen receptor-regulated process in-vitro. Endocrinology. 1981;109(4):1303–5.
111. Vendola KA, Zhou J, Adesanya OO, et al. Androgens stimulate early stages of follicular growth in the primate ovary. J Clin Invest. 1998;101(12):2622–9.
112. Bosdou JK, Venetis CA, Kolibianakis EM, et al. The use of androgens or androgen-modulating agents in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. Hum Reprod Update. 2012;18(2):127–45.
113. Sunkara SK, Pundir J, Khalaf Y. Effect of androgen supplementation or modulation on ovarian stimulation outcome in poor responders: a meta-analysis. Reprod Biomed Online. 2011;22(6):545–55.
114. Kim CH, Howles CM, Lee HA. The effect of transdermal testosterone gel pretreatment on controlled ovarian stimulation and IVF outcome in low responders. Fertil Steril. 2011;95(2):679–83.
115. Massin N, Cedrin-Durnerin I, Coussieu C, et al. Effects of transdermal testosterone application on the ovarian response to FSH in poor responders undergoing assisted reproduction technique–a prospective, randomized, double-blind study. Hum Reprod. 2006;21(5):1204–11.
116. Ozmen B, Sonmezer M, Atabekoglu CS, et al. Use of aromatase inhibitors in poor-responder patients receiving GnRH antagonist protocols. Reprod Biomed Online. 2009;19(4):478–85.
117. Barrenetxea G, Agirregoikoa JA, Jimenez MR, et al. Ovarian response and pregnancy outcome in poor-responder women: a randomized controlled trial on the effect of luteinizing hormone supplementation on in vitro fertilization cycles. Fertil Steril. 2008; 89(3):546–53.
118. Berkkanoglu M, Isikoglu M, Aydin D, et al. Clinical effects of ovulation induction with recombinant follicle-stimulating hormone supplemented with

recombinant luteinizing hormone or low-dose recombinant human chorionic gonadotropin in the midfollicular phase in microdose cycles in poor responders. Fertil Steril. 2007;88(3):665–9.
119. Ferraretti AP, Gianaroli L, Magli MC, et al. Exogenous luteinizing hormone in controlled ovarian hyperstimulation for assisted reproduction techniques. Fertil Steril. 2004;82(6):1521–6.
120. Ruvolo G, Bosco L, Pane A, et al. Lower apoptosis rate in human cumulus cells after administration of recombinant luteinizing hormone to women undergoing ovarian stimulation for in vitro fertilization procedures. Fertil Steril. 2007;87(3):542–6.
121. Wiser A, Gonen O, Ghetler Y, et al. Addition of dehydroepiandrosterone (DHEA) for poor-responder patients before and during IVF treatment improves the pregnancy rate: a randomized prospective study. Hum Reprod. 2010;25(10):2496–500.
122. Mochtar MH, Van der V, Ziech M, et al. Recombinant Luteinizing Hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. Cochrane Database Syst Rev. 2007;2:CD005070.
123. Goswami SK, Das T, Chattopadhyay R, et al. A randomized single-blind controlled trial of letrozole as a low-cost IVF protocol in women with poor ovarian response: a preliminary report. Hum Reprod. 2004; 19(9):2031–5.
124. Fabregues F, Penarrubia J, Creus M, et al. Transdermal testosterone may improve ovarian response to gonadotrophins in low-responder IVF patients: a randomized, clinical trial. Hum Reprod. 2009;24(2):349–59.
125. Barad D, Brill H, Gleicher N. Update on the use of dehydroepiandrosterone supplementation among women with diminished ovarian function. J Assist Reprod Genet. 2007;24(12):629–34.
126. Garcia-Velasco JA, Moreno L, Pacheco A, et al. The aromatase inhibitor letrozole increases the concentration of intraovarian androgens and improves in vitro fertilization outcome in low responder patients: a pilot study. Fertil Steril. 2005;84(1):82–7.
127. Schoolcraft WB, Surrey ES, Minjarez DA, et al. Management of poor responders: can outcomes be improved with a novel gonadotropin-releasing hormone antagonist/letrozole protocol? Fertil Steril. 2008;89(1):151–6.
128. Yarali H, Esinler I, Polat M, et al. Antagonist/letrozole protocol in poor ovarian responders for intracytoplasmic sperm injection: a comparative study with the microdose flare-up protocol. Fertil Steril. 2009;92(1):231–5.

Cryopreserved Oocyte Banking: Its Prospects and Promise

11

Kathryn J. Go, Zsolt Peter Nagy, and Ching-Chien Chang

11.1 Introduction

It is difficult to imagine in vitro fertilization (IVF) and assisted reproductive technologies (ART) without cryopreservation. The science and craft of freezing cells and tissues with preservation and resumption of their biological functions after thawing results from the research of a host of investigators. Much is owed to their contributions in defining cryopreservative and warming solution formulations and description of cell- and tissue-specific methodologies [1].

Both patients and practitioners of ART have been unique beneficiaries of the ability to cryopreserve reproductive cells. The use of frozen sperm was broached as early as 1950 [2] and ART with both frozen autologous and donor sperm is a long-standing treatment option for infertility. Cryopreservation of zygotes, early cleavage stage embryos, and blastocysts is integral to allowing patients to maximize and optimize a single cycle of ovulation induction for ART.

K.J. Go, Ph.D. (✉)
Reproductive Science Center, University of Massachusetts, Lexington, MA 02421, USA

Department of Obstetrics and Gynecology, University of Massachusetts Medical School, Worcester, MA, USA
e-mail: Kathy.go@integramed.com

Z.P. Nagy, Ph.D. • C.-C. Chang, Ph.D.
Reproductive Science Center, University of Massachusetts, Lexington, MA 02421, USA

The cryopreservation of human eggs, in contrast, has been elusive [3], but significant strides in technique have been made, yielding the desired characteristics of consistently high rates of post-thaw survival, fertilization, embryo development, and implantation. It is a testament to this achievement that the potential for cryopreserved egg banking is addressed in this biennial review. The recent withdrawal of the qualifier, "experimental," from oocyte freezing by the American Society of Reproductive Medicine [4] may hasten the rapid acquisition of this technology by more ART laboratories and augment the range of reproductive options by both fertile and subfertile women and those using third-party reproductive strategies for family building.

11.2 The Unique Challenges of Egg Cryopreservation

Success of oocyte freezing, i.e., implantation and pregnancy, was reported early in the history of ART [5]—only 3 years after the report of the first successful embryo thaw [6]—inspiring the hope that oocytes would lend themselves to the prevailing slow-cooling methods for cryopreservation for cleavage stage embryos. The advantages of being able to freeze the full range of reproductive cells, i.e., *both* types of gametes as well as embryos, were enormous. While very encouraging results followed [7–14], oocyte freezing proved challenging and was not integrated into routine practice at the same trajectory as cleavage-stage

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_11,

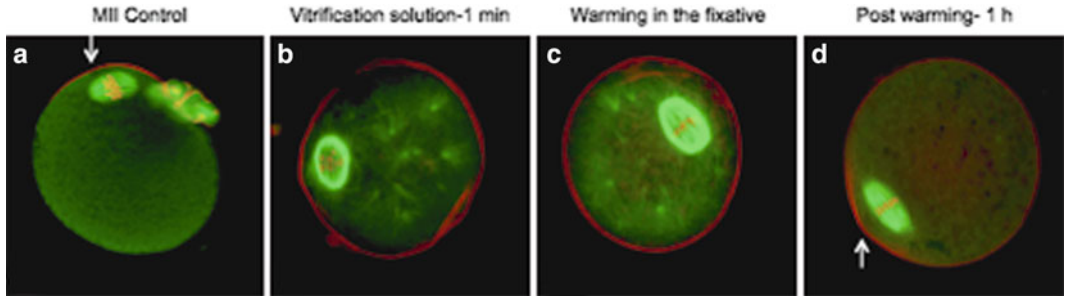

Fig. 11.1 Influence of the oocyte vitrification on cytoskeleton structures of mouse oocytes. Confocal images of microtubules (*Green*), microfilaments (*Red*) with chromatin (*Red*), and merge of representative oocytes before vitrification (**a**), treated with vitrification solution (containing 15 % DMSO and 15 % ethylene glycol, and 0.5 M sucrose) for 1 min at RT (**b**), warmed the vitrified oocyte directly into the fixative (**c**), and an oocyte was cultured for 1 h after warming (**d**). After oocytes warmed, it displays that stabilized MII spindle with chromosomes and the adjacent microfilament-rich domains (*arrow*) resembling to oocytes prior to the vitrification process

embryos and later blastocysts. The most prevailing challenge was at the level of survival, requiring up to 100 oocytes for a single successful pregnancy.

Some unique factors must be surmounted in freezing mature (Metaphase II) oocytes. Human oocytes (a) are large cells presenting the challenge of high intracellular water volume; (b) have a complex intracellular architecture comprised of cortical granules, organelles, and microtubules that must be protected [15–18]; and (c) are arrested in meiosis thereby requiring special care to avoid disruption of the spindle and its chromosomes (Fig. 11.1) [7, 8, 19, 20]. In addition, the membrane properties of an oocyte are significantly different than the similarly sized zygote, possibly attributable in part to aquaporin, a protein channel that can provide transport of water and other solutes through the oolemma.

These translated to the technical hurdles of adequate dehydration, protection from cryopreservative toxicity, and conservation of cellular integrity at warming. Postthaw survival would be measured not only in recovery of an intact, hydrated cell but also an egg that could be fertilized, resuming meiosis without risk of aneuploidy from a disrupted spindle, and capable of normal developmental progression.

Egg cryopreservation required the confluence of two techniques to realize its clinical application: vitrification and ICSI. The adoption of vitrification into ART—brief exposure to high cryoprotectant concentration with the use of "open" cryopreservation carriers that allowed maximal cooling rates—was catalytic to the rapid development of egg cryopreservation methods [21, 22]. Careful formulation of equilibration and vitrification media was coupled with determination of optimal equilibration times to avoid the toxicity from exposure to high concentration of cryoprotectants. Open systems, such as OPS (open pulled straw), CryoTops, CryoLocks, CryoLeafs, CryoLoops, and others, as well as several closed carriers, in conjunction with these carefully designed techniques for warming, yielded the desired high rates of egg recovery and survival [23–26].

To counter any changes in the physical characteristics of the zona pellucida that might impede sperm binding and/or penetration, intracytoplasmic sperm injection (ICSI) has generally been accepted as the optimal approach to insemination [27, 28] although some studies reported normal fertilization of frozen-thawed eggs with conventional insemination [8, 9]. While minimizing the risk of fertilization failure, ICSI also allows close appraisal of the postthaw oocyte as appearance of the ooplasm, membrane resistance and dynamics of the sperm injection can be reliable markers or predictors of oocyte quality [9, 29].

11.3 The Clinical Utility of Cryopreserved Egg Banking

The application of oocyte cryopreservation can fulfill several therapeutic purposes. Two of the most anticipated are autologous fertility preservation and the development of donor oocyte banks [30, 31].

11.3.1 Autologous Oocyte Banking

To forestall the inevitability of declining ovarian reserve and oocyte quality with age, women can elect to undergo one or more cycles of ovulation induction with freezing of the oocytes for later use [32]. Oocyte freezing may thus relieve the pressure of the inexorable advance of the biological clock and ameliorate the disappointment of women in their waning reproductive years who undergo IVF with reduced odds of pregnancy [33].

Fertility preservation may take a more pressing form, as when young women confront loss of ovarian function from cancer treatment. A chance for reproductive potential is preserved through oocyte freezing if ovulation induction and retrieval are not counter-indicated [34].

In advance of the hormonal and surgical interventions for gender reassignment, women can freeze their oocytes, preserving the opportunity reproduction with their genetic material.

With the admission of military women to combat roles, oocyte freezing may provide some insurance against fertility loss from grievous wounds.

For patients who wrestle with the implications of creating more embryos than needed for embryo transfer and cycle completion and the thorny issues of their disposition if these embryos are not required or desired for future transfers, oocyte cryopreservation allows allocation of some oocytes to be used for insemination and others to be stored [35]. In alleviating some of the ethical concerns of cryopreserved embryos, oocyte freezing and banking may be a welcomed adjunct to IVF. Somewhat unexpectedly, the option of cryopreserving "extra" eggs (not used for insemination) and avoidance of excess embryos is currently one of the most frequent applications of oocyte cryopreservation.

11.3.2 Donor Oocyte Banking

One of the many dividends of ART has been the opportunity for individuals to reproduce using donor oocytes, widening the reproductive horizon for women whose fertility was imperiled by diminished ovarian function or loss. IVF with donor oocytes became a well-established treatment but was offered primarily with "fresh" oocytes until recently. While this was a practical treatment model, there were some disadvantages.

Cycle synchronization between oocyte donor and oocyte recipient had to be achieved. Because the schedules of two individuals (donor and recipient) required accommodation, convenience to the recipient was not a hallmark of this approach. Compared to sperm banks, the array of desired characteristics and ethnicities was limited to the donors provided by agencies specializing in their recruitment or individual IVF centers who developed their own donor catalogues. In addition, the safety of fresh oocyte donation, despite rigorous donor screening and testing, may not be at the same level as cryopreserved donor oocytes, in which retesting of donors for infectious agents after 6 months is an option, completely analogous to the standard for sperm donors.

While the clinical efficacy of fresh oocyte donation in yielding pregnancies and live births is evident as reflected in the outcomes published annually by the Society of Assisted Reproductive Technology (SART) and the Centers for Disease Control and Prevention (CDC), some potential patients may be daunted and discouraged by the need for cycle synchronization between recipient and donor that may result in treatment delay, the lack of an appropriate oocyte donor, lapses in donor compliance that may lead to cycle cancellation, and a prolongation of disappointment and frustration.

Donor oocyte banks can provide (a) wide selection of donors with desired phenotypic characteristics from a catalogue; (b) the availability of a relatively rare donor, e.g., of mixed ancestry; and (c) the convenience of commencing IVF treatment once the donor oocytes are selected and obtained by the clinic. In addition, IVF clinics would be relieved of the considerable financial and administrative burdens of recruiting, screening, and maintaining their own donors and may be able to increase the number of donor oocyte cycles using donor oocyte banks.

Access, variety, immediate availability, and comparable pregnancy outcomes to fresh egg donation (Table 11.1): these are all features that would render donor oocyte banks the same successful enterprise that sperm banks have proven

Table 11.1 The IVF treatment outcome of using vitrified donor oocytes for recipients (vitrified donor oocytes provided by My Egg Bank North America and recipients treated at Reproductive Biology Associates, Atlanta, GA)

Outcome	
Donation cycle	119
Age of donors (years)	26.3 ± 2.7
Recipient cycles	436
Age of recipient (years)	41.4 ± 4.4
Total oocyte warmed (per recipient)	2,656 (6.09 ± 1.65)
Total oocyte survived (%)	2,453/2,656 (92.3 %)
Total oocyte fertilized (%)	2,161/2,453 (88.0 %)
Good-quality embryo on day 3 (per fertilized oocyte)[a]	1,501/2,161 (69.4 %)
Blastocyst formation rate (per embryo subjected to extended culture)	1,482/2,089 (70.9 %)
Total number of embryo transferred (per recipient)	592 (1.36 ± 0.48)
Total number of embryo revitrified	1,054 (2.42 ± 1.23)
Clinical pregnancies (%)	285/436 (65.3 %)
Total number of implantation (%)	352/592 (59.4 %)
Total number of ongoing pregnancy	279
Total number of recipient delivered[b]	168
Total number of infants born	219 (103 female and 116 male)

[a]According to SART morphological assessment for embryo grading system

[b]There were still 111 recipients with ongoing pregnancy who have not delivered yet by the time this manuscript was prepared

to be for decades. Additionally, acquiring oocytes from a cryobank is financially more affordable than fresh oocyte donation, mainly because the cost of a single oocyte donor is distributed among several recipients.

11.3.3 Comparison with and Contrast to Sperm Banking

Although donor oocyte banks may now emerge, the considerable difference between oocyte and sperm banking merits attention. Sperm banks have the luxury of evaluating a high number of candidates who, despite normal seminal parameters of sperm concentration, motility, and morphology, may not produce the minimal number of motile sperm post-thaw and are declined. The rate of acceptance to be a sperm donor at a commercial sperm bank can be restrictive without limiting the creation of inventory. Owing to this ability to be selective and eliminate donors whose sperm are cryo-sensitive, many sperm banks are able to offer a warranty for each sample, guaranteeing a minimum of total motile sperm post-thaw, a feature that augments their attractiveness to clients.

Oocyte banking does not easily make this accommodation for donor exclusion. Once candidates are screened and accepted, and reasonable ovarian response to controlled hyperstimulation is achieved with retrieval and freezing of mature eggs, knowledge of the oocytes' quality must be obtained empirically. A "test thaw" will reveal if an egg can be recovered structurally intact, but it will be only after ICSI, appraisal of embryo development and transfer, that the "quality," i.e., the ultimate ability of the frozen oocyte to advance to embryo implantation and clinical pregnancy can be determined. For this reason, donor oocyte banks can only *retrospectively* withdraw a suboptimal donor after appropriate review.

11.4 A Model for a Donor Oocyte Bank

A donor oocyte bank represents not only a scientific and medical resource to assist women and couples in achieving pregnancy and live birth, but it is also a novel business model. As such, an effective and successful oocyte bank demands the appropriate infrastructure, support, and maintenance for its organization and establishment, production of consistent positive outcomes to build a reputation for service, reliability, and quality, and to evolve as patients and the marketplace suggest or dictate. Some of the required elements of a donor oocyte bank are the following:

1. A well-designed and executed donor recruitment program.
2. An efficient and effective screening process for applicants who wish to be oocyte donors.

3. A qualified mental health professional who can administer the appropriate instruments required to assess the donor's understanding of gamete donation and its potential ramifications.
4. A prescribed methodology for ovulation induction of the oocyte donors to achieve consistency in this critical phase of the process.
5. A validated, reproducible method for oocyte vitrification and warming must be applied and the embryologists of the oocyte bank and the recipient laboratories must be carefully and rigorously trained in the vitrification and warming methods, respectively. This will ensure the consistency and quality control leading to optimal outcomes and be an integral part of the foundation for the bank's reputation and success.
6. A vigorous quality control program for the reagents and materials used in the oocyte bank, completely analogous to that of an ART laboratory.
7. A team of administrators to manage and organize the communication with and information from applicants, accepted donors, cycling donors, and their respective recipients.
8. A database that can track donor oocyte acquisition, distribution, clinical use, and clinical outcomes.
9. A database manager who will provide oversight on donor outcomes, e.g., to ensure that maximal cycle number by a given donor is not exceeded or that an underperforming donor is reviewed.
10. Excellent communication and coordination between the oocyte bank and its recipient laboratories.
11. A full understanding of and compliance with all regulations governing reproductive cells and tissue, i.e., those of the Food and Drug Administration (FDA), the Society of Assisted Reproductive Technology (SART), and individual state requirements.
12. A mission statement that includes a commitment to the welfare of both donors and recipients.

11.5 The Future of Oocyte Banking

A reliable method of cryopreserving oocytes allows patients to freeze and store their own oocytes to ameliorate loss of fertility through age, disease, or ovarian loss or injury. An additional application is assisting patients who wish to avoid creation of supernumerary embryos through allocation of some oocytes to IVF and some to freezing. This strategy ensures that every oocyte is clinically used and maximizes the potential of each treatment cycle while avoiding the difficult decisions and controversies that may surround cryopreserved zygotes or embryos.

The ability to offer cryopreserved donor oocytes, i.e., through a donor oocyte bank, is exceedingly attractive from a convenience-to-patient perspective and the ability to initiate therapy rapidly. Donor oocyte banks are a significant venture, requiring medical, scientific, business and administrative skill and strong communication and organization. Their emergence may impart greater urgency to the effort to create a central oocyte donor registry to keep an accounting of how many cycles a specific oocyte donor undergoes and how many offspring result from her donations [36–38].

As cryopreserved oocyte banking becomes established as the newest ART, it may be important to consider how it will evolve. A new generation of potential users of this technique, whether for autologous fertility preservation or as donors or recipients, brings its own expectations and values. This is a generation accustomed to rapidly developing medical technology and fully expectant of virtually instant communication, high levels of social connectivity through electronic media, and robust access to information.

Just as IVF, embryo cryopreservation, assisted hatching, ICSI, and embryo biopsy for preimplantation diagnosis fulfilled the family-building ambitions of patients in the 1980s and 1990s, oocyte cryopreservation and its benefits of fertility preservation and donor oocyte banking brings greater prospects and maybe even the *promise* for family building in the twenty-first century.

References

1. Mazur P. Freezing of living cells: mechanisms and implications. Am J Physiol. 1984;247(3 Pt 1): C125–42.
2. Farris EJ. Human fertility and problems of the male. White Plains, NY: The Author's Press; 1950.
3. Maher B. Little consensus on egg freezing. Nature. 2007;449(7165):958.
4. The practice committees of the american society for reproductive medicine and the society for assisted reproductive technology. Mature oocyte cryopreservation: a guideline. Fertil Steril. 2013;99:37–43.
5. Chen C. Pregnancy after human oocyte cryopreservation. Lancet. 1986;1(8486):884–6.
6. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature. 1983;305(5936):707–9.
7. Gook DA, Osborn SM, Johnston WI. Cryopreservation of mouse and human oocytes using 1,2-propanediol and the configuration of the meiotic spindle. Hum Reprod. 1993;8(7):1101–9.
8. Gook DA, Osborn SM, Bourne H, et al. Fertilization of human oocytes following cryopreservation; normal karyotypes and absence of stray chromosomes. Hum Reprod. 1994;9(4):684–91.
9. Gook DA, Schiewe MC, Osborn SM, et al. Intracytoplasmic sperm injection and embryo development of human oocytes cryopreserved using 1,2-propanediol. Hum Reprod. 1995;10(10):2637–41.
10. Fabbri R, Porcu E, Marsella T, et al. Human oocyte cryopreservation: new perspectives regarding oocyte survival. Hum Reprod. 2001;16(3):411–6.
11. Porcu E. Oocyte cryopreservation. In: Gardner DK, Weissman A, Howles CM, Shoham Z, editors. Textbook of assisted reproductive techniques: laboratory and clinical perspectives. London: Martin Dunitz; 2001. p. 233–42.
12. Borini A, Bonu MA, Coticchio G, et al. Pregnancies and births after oocyte cryopreservation. Fertil Steril. 2004;82(3):601–5.
13. Boldt J, Tidswell N, Sayers A, et al. Human oocyte cryopreservation: 5-year experience with a sodium-depleted slow freezing method. Reprod Biomed Online. 2006;13(1):96–100.
14. Coticchio G, De Santis L. Slow freezing of oocytes. In: Nagy ZP, Varghese AC, Agarwal A, editors. Practical manual of in vitro fertilization. New York: Springer; 2012. p. 509–15.
15. Schalkoff ME, Oskowitz SP, Powers RD. Ultrastructural observations of human and mouse oocytes treated with cryopreservatives. Biol Reprod. 1989;40(2):379–93.
16. Sathananthan AH. Paternal centrosomal dynamics in early human development and infertility. J Assist Reprod Genet. 1998;15(3):129–39.
17. Ghetler Y, Skutelsky E, Ben Nun I, et al. Human oocyte cryopreservation and the fate of cortical granules. Fertil Steril. 2006;86(1):210–6.
18. Gualtieri R, Iaccarino M, Mollo V, et al. Slow cooling of human oocytes: ultrastructural injuries and apoptotic status. Fertil Steril. 2009;91(4):1023–34.
19. Van Blerkom J, Davis PW. Cytogenetic, cellular, and developmental consequences of cryopreservation of immature and mature mouse and human oocytes. Microsc Res Tech. 1994;27(2):165–93.
20. Bromfield JJ, Coticchio G, Hutt K, et al. Meiotic spindle dynamics in human oocytes following slow-cooling cryopreservation. Hum Reprod. 2009;24(9): 2114–23.
21. Rall WF, Fahy GM. Ice-free cryopreservation of mouse embryos at -196 degrees C by vitrification. Nature. 1985;313(6003):573–5.
22. Liebermann J, Nawroth F, Isachenko V, et al. Potential importance of vitrification in reproductive medicine. Biol Reprod. 2002;67(6):1671–80.
23. Kuwayama M, Vajta G, Kato O, et al. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod Biomed Online. 2005;11(3): 300–8.
24. Lucena E, Bernal DP, Lucena C, et al. Successful ongoing pregnancies after vitrification of oocytes. Fertil Steril. 2006;85(1):108–11.
25. Vajta G, Nagy ZP. Are programmable freezers still needed in the embryo laboratory? Review on vitrification. Reprod Biomed Online. 2006;12(6):779–96.
26. Cobo A. Oocyte vitrification. In: Nagy ZP, Varghese AC, Agarwal A, editors. Practical manual of in vitro fertilization. New York: Springer; 2012. p. 523–8.
27. Porcu E, Fabbri R, Seracchioli R, et al. Birth of a healthy female after intracytoplasmic sperm injection of cryopreserved human oocytes. Fertil Steril. 1997; 68(4):724–6.
28. Chen SU, Lien YR, Chen HF, et al. Observational clinical follow-up of oocyte cryopreservation using a slow-freezing method with 1,2-propanediol plus sucrose followed by ICSI. Hum Reprod. 2005;20(7): 1975–80.
29. Palermo G, Joris H, Devroey P, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1992;340(8810):17–8.
30. Nagy ZP, Chang CC, Shapiro DB, et al. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. Fertil Steril. 2009;92(2): 520–6.
31. Cobo A, Meseguer M, Remohi J, et al. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. Hum Reprod. 2010;25(9):2239–46.
32. Cobo A, Domingo J, Perez S, et al. Vitrification: an effective new approach to oocyte banking and preserving fertility in cancer patients. Clin Transl Oncol. 2008;10(5):268–73.

33. Homburg R, van der Veen F, Silber SJ. Oocyte vitrification – women's emancipation set in stone. Fertil Steril. 2009;91(4 Suppl):1319–20.
34. Almog B, Azem F, Gordon D, et al. Effects of cancer on ovarian response in controlled ovarian stimulation for fertility preservation. Fertil Steril. 2012;98(4): 957–60.
35. Levi Setti PE, Albani E, Novara PV, et al. Cryopreservation of supernumerary oocytes in IVF/ICSI cycles. Hum Reprod. 2006;21(2):370–5.
36. Elster NR, Braverman AM. The future is now: a voluntary gamete donor registry is feasible. DePaul J Health Care L. 2009;(12):195–201.
37. Benward J, Braverman AM, Galen B. Maximizing autonomy and the changing view of donor conception: The creation of a national donor registry. DePaul J Health Care L. 2009;(12):225–40.
38. Braverman, AM. How the internet is reshaping assisted reproduction: from donor offspring registries to direct-to-consumer genetic testing. Minn J of Law, Science and Technology. 2012;11:477–96.

Reproductive Tourism

12

Raphael Ron-El

12.1 Introduction

Assisted reproductive technologies (ART) may raise reproductive situations that create ethical issues that result in legislative action. From the beginning, advances in these technologies used for the treatment of infertility problems have created ethical problems that may eventually emerge after a certain delay. Ethical conditions may result in legislative rules that are typically decided in democracies by politicians who pass these laws. Therefore, a compromise between politics and ethics should be attempted, meaning that the majority may impose their ethical attitude on the minority. However, the majority should do it very cautiously, respecting the different moral positions leaving certain moral liberalism to the minority [1].

What does "Liberalism" mean in sense of reproductive treatments? Letting those who wish to obtain their desired treatment outside the boundaries of their own country, as long such treatment is achievable [1]. There is no unified culture in the world, even in the Western world, and not even among the different countries of the European Union. There is no predetermined core of substantive common values among these different cultures. This diversity is to be valued and does not represent a limitation. The wish for homogeneous ethical values denies the richness of cultural, political, and ethical differences. It also impedes progress toward better regulation [1].

Along with the principle of "Liberalism" and the rights of the minority to achieve their wish to have their child by treatment outside their own country, it should be discussed whether citizens in a democracy have the right to seek treatment abroad when it is legally forbidden in their own country? This complicated question has been argued by different ethical and professional organizations during the past several years. The European Society of Human Reproduction and Embryology (ESHRE) has summarized the issue of Cross-Border Reproductive Care (CBRC) in the "ESHRE Task Force on Ethics and Law 15" [2]. In addition to other issues, this task force has addressed whether a patient has the right to get treatment abroad when it is legally forbidden in their own country, stating "Recent developments have attributed more value to reproductive autonomy, therefore, transgression [of local legislative restrictions] is justified as long as safety, efficacy and welfare of the patient and future child is considered" [2]. This cautious principle given by one of the leading societies in the field of reproduction opens the official door for medical tourism, a topic that was unofficial for a long period previously.

R. Ron-El, M.D. (✉)
Fertility and IVF Unit, Department of Obstetrics & Gynecology, Assaf Harofeh Medical Center, Sackler Medical School, Tel Aviv University, Tel Aviv, Israel
e-mail: rafirnel@gmail.com

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_12,

12.2 What Should "Reproductive Tourism" Be Called?

Since the whole idea of people traveling outside their countries to seek medical aid was and still is not well accepted by all the public, the description of the phenomenon has substantial importance. There is controversy regarding the appropriate title and description for "Reproductive Tourism." Appropriate terminology is important in framing the semantics of public debates and policy making.

The first definition of transborder reproductive care was created by the ethicist Guido Pennings, who called it "medical tourism" [3]. Since the phenomenon of medical tourism has increased in many fields of medicine, Pennings suggested 2 years later that the term "reproductive tourism" be used to differentiate patients seeking assistance in reproduction outside the borders of their own countries from other patients seeking care for treatment in other medical field [1].

Mattoras as well as Inhorn and Patrizio were of the opinion that the description "reproductive tourism" implies fun, holidays, and leisure. It sounds like a "gimmick" that could create a mockery of the medical condition and suffering of infertile people who are seeking medical care [4, 5]. These authors have suggested the term "reproductive exile." The term exile reflects the forced removal from your native country or voluntary absence to seek medical treatment. Where medical treatment is required because of legislative restrictions, the term "exile" described may most accurately reflect the feeling of the patient.

The definition "cross-border reproductive care" (CBRC) was suggested again by Pennings to avoid the negative connotation of tourism [6]. The title CBRC is an objective and descriptive one and does not involve feelings or connotation. Cross-Border Reproductive Care also coincides with the term "cross-border health care", which was used by the Commission of the European Communities (2004) [7].

Although the CRBC is well respected by most sectors, some concern has been raised regarding this approach for reproduction options, including an article by Rose and Rose (2003) in *The Guardian* newspaper [8]. They protested against the inequality of access to such treatment options. Although it is possible for patients from highly regulated countries to go to less regulated countries, access to such treatment clearly requires resources that may not be available to the average citizen. Therefore, it may be considered unjust and discriminatory.

12.3 Rationale for Reproductive Tourism

Reproductive tourism is most commonly accessed because of the lack of options for treatments in the country of origin of the patients. An argument for CBRC can be made when treatment is prohibited because the procedures are locally prohibited from ethical or religious limitations such as donation of gametes or surrogacy; when characteristics of the treatment unfit parenthood such as postmenopausal woman or homosexuals. If a procedure in some countries is estimated to be unsafe such as oocyte freezing or cytoplasmic transfer. Or treatment is unavailable due to lack of expertise such as preimplantation diagnosis (PGD). Long waiting lists to access reproductive treatments or excessive treatment cost in their country of origin are other reasons to access reproductive tourism. Finally, individuals may wish to access reproductive options to maintain privacy from family or friends and thereby seek care outside their country (Table 12.1).

Table 12.1 The main reasons for reproductive tourism

Status in the country of origin	Examples
Treatment is prohibited due to ethically or religious unaccepted procedure	Donor gametes, gendering
Characteristics unfit to parenthood	Postmenopausal, gay orientation
Procedure is considered unsafe	Oocyte freezing, cytoplasmic transfer
Unavailable treatment due to lack of expertise	PGD
Long waiting list	Egg donation
Cost too high	
Individuals who wish to keep their privacy	Donor gametes, any ART

12.4 Forbidden Procedures in Different Countries

Table 12.2 shows the forbidden procedures across Europe [9]. Access to ART is forbidden for single women and lesbians in France (Table 12.2). The Netherlands will not permit ART treatment to be performed in women beyond the age of 41 years. In Turkey, female patients more than 40 years of age cannot be treated with assisted reproduction. Sperm donation is not possible in Turkey and is not permitted in France for single women and lesbians. Oocyte donation is not permitted in Germany, Norway, and Turkey. Testicular biopsy and testicular aspiration were prohibited until recently in The Netherlands and are now limited to only two clinics. Since 2007, such treatments are only considered as part of a research program. Preimplantion genetic diagnosis (PGD) is only allowed in The Netherlands at one center (Maastricht) and in Germany it can only be performed on polar bodies. Surrogacy is prohibited in Germany, Norway, Spain, and Turkey; embryo freezing is forbidden in Italy and Germany.

Table 12.2 Forbidden procedures across Europe

Forbidden procedures	Countries	Limitations
Access to ART	France NL Turkey	Single women, lesbians Age>41 Age>39
Sperm donation	France Turkey	Single women, lesbians
Oocyte donation	Germany Italy Norway Turkey	
TESE/PESA	NL	Limited to only two clinics Since 2007—part of research program
PGD	Germany NL	Permitted only in PB Except for: one center (Maastricht)—BRCA
Surrogacy	Germany Norway Spain Turkey	
Embryo freezing	Italy Germany	

Donation of gametes and surrogacy is forbidden in most Islamic countries. In the USA, regulations vary from state to state. In some states, surrogacy is permitted, while in others it is forbidden. More recently, some countries have permitted gamete donation only when the donor is known to the recipient or can be known to the child born following the gamete donation. This option is not accepted by some gamete recipients who prefer anonymity of their donors and so they may prefer reproductive tourism over the possibility to be treated in their own country.

12.5 Frequency of Cross-Border Reproductive Care

No routine collection of data allow accurate quantification of the extent of medical tourism, so there is a lack of information about the type, quality, and quantity of CBRC, which is performed. Medical tourism is estimated to represent 7–10 % of all assisted reproductive treatments worldwide. This speculated estimation was provided by John Collins from Canada, in 2009, during the "First International Meeting of Cross-Border Reproductive Care" in Ottawa [10].

Belgium is the only country in which information about CBRC performance within its border is routinely available. During the year 1999, 30 % of the ART cycles, 60 % of the egg recipients, and 50 % of the PGD treatment cycles were done on non-Belgian patients [11].

In 2003, 20 % of 11,245 ART cycles were performed on patients outside Belgium, 15 % of 14,795 in 2004, and 18 % of 95,177 cycles during the years 2005–2007 [11]. Figure 12.1 shows the number of foreign patients per nationality coming to Belgium during the years 2005–2007 (Fig. 12.1) [9]. Figure 12.2 shows the distribution of patients seeking treatment in Belgium according to treatment and nationality (Fig. 12.2) [9].

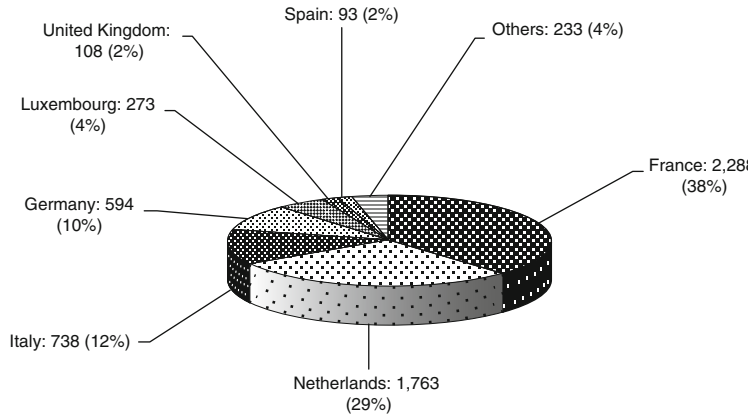

Fig. 12.1 Number of foreign patients per nationality treated in Belgium from 2005 to 2007. The total number of foreign patients treated in that time period was 6,090 (reproduced with permission from Pennings et al. [9])

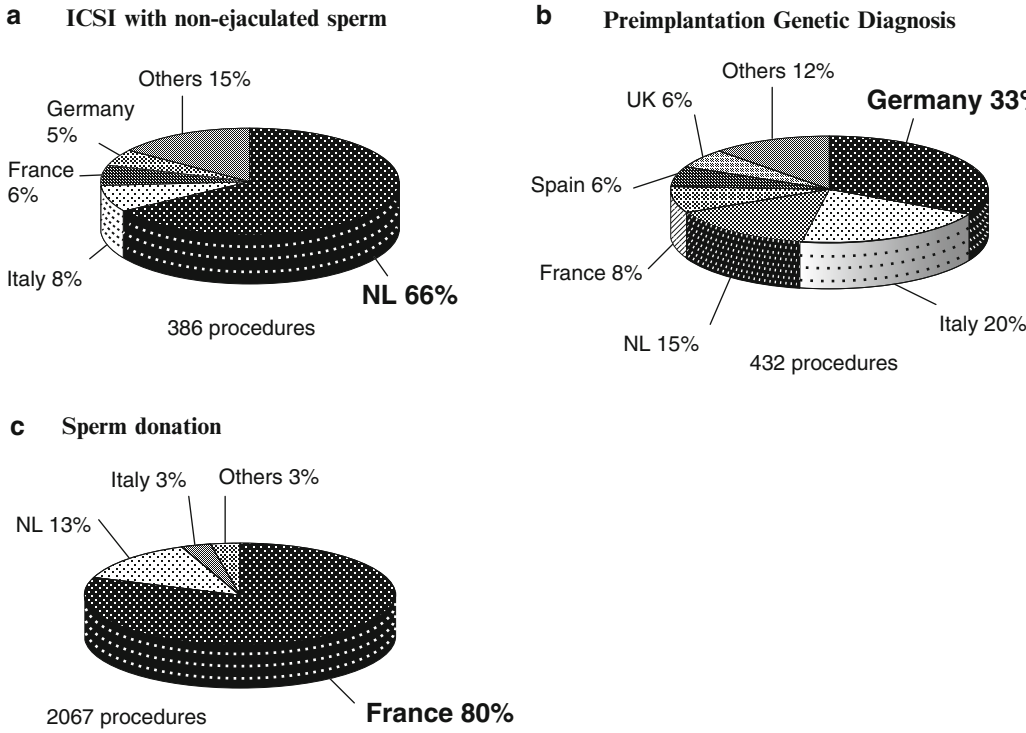

Fig. 12.2 Foreign patients treated in Belgium from 2005 to 2007 according to the type of treatment performed (reproduced with permission from Pennings et al. [9])

12.6 Medical and Ethical Concerns in Reproductive Tourism

Over years and with the increasing use of CBRC, medical and ethical concerns became more evident and have created increasing discussion in published literature and in scientific meetings.

The University College Hospital in London has reported on the impact of CBRC on maternity services [12]. The authors have demonstrated that high-order multiple pregnancies (≥3) have dramatically increased during the years 1996–2006, associated with British patients being treated with IVF services outside of the UK. Out of 56 women seen with high-order pregnancies at the

University College Hospital, another 20 women with such pregnancies were seen for couples treated outside the UK. This caused a 36 % increased frequency of high-order multiple pregnancies during this period of time. In essence, the strict regulations on the number of the transferred embryos in the country of origin may frequently be circumvented if treatment is performed outside the country's borders.

The main ethical problems in the field of reproductive tourism are related to egg donation and surrogacy, which are commonly performed by CBRC. Egg donation involves two main problems, the financial—trade one and the risk of exploitation of vulnerable individuals in poor countries. The European Parliament resolution on the trade in human egg cells (sitting of 10.03.2005) stated that "Harvesting of egg cells poses a high medical risk to the life and health of women, resulting from hyperstimulation of the ovaries" [13]. The parliament "Wishes to see egg cell donation, like organ donation generally, strictly regulated in order to protect both donors and recipients and to tackle all forms of human exploitation." Therefore, "Article 12 makes clear that payment other than compensation, for cell and tissue donations in Europe is not accepted and that cells and tissues must not as such be a subject to trade."

They continue with their statement stating that "This provision leaves responsibility for authorizing and setting the levels of compensation within the framework of the Directives in question to the member state." Therefore, it is understandable that compensation to the egg donor vary from country to country. For instance, the following rates of payments appear in official places like in the Web site of "Human Fertilisation Embryology Authority" (HFEA) mentions a compensation of £55 per day till a maximum of £250. The Israeli law of egg donation mentions the compensation of 10,000 NIS (equivalent to 2000 €) to the donor, which has to be paid by the recipient via the administration of the hospital [14, 15]. The expenses of the treatment itself are covered by the medical insurance. These are the only official fees mentioned written. The compensations in the different countries normally will vary between some hundreds of Euros (mainly in the Eastern European Countries) up to couple of thousands of US Dollars in the USA.

The most concerning issue about "compensation to the egg donor" is the difference between "compensation" and "payment." The expression "compensation" may relax our or societies' consciousness that excess payment occurs, which may unduly influence donor's motivation to participate in oocyte donation. On the other hand, altruism may not provide adequate potential oocyte donors to provide gametes.

12.7 Recent Trends in Reproductive Tourism

The activity of oocyte donation and surrogacy has been concentrated in two geographical areas. Egg donation is commonly performed in centers across Eastern Europe with no information about the magnitude of the phenomenon. Far fewer cycles of egg donation are performed not only in Western Europe, mainly in Spain, but also in Belgium, Greece, UK, and some other countries to a small extent. Some states in the USA also permit and perform egg donation. Since the introduction of vitrification of oocytes with a high survival rate after their warming, egg banks have been created in large centers that perform egg donation. This fact enables couples to bypass synchronization of the recipient with the treatment cycle of the donor. It also permits the recipient to choose the timing for selection of a specific donor that she and/or the couple desires.

Surrogacy is rapidly increasing in frequency in India and Thailand. In India, commercial surrogacy was legalized in 2002 to promote reproductive tourism [16]. Since many countries in Europe do not permit surrogacy, and UK law dictates that surrogacy must be driven by altruism, many patients find their way to India where surrogacy is accessible and relatively cheap. The Indian Council of Medical Research tries to regulate the centers but permits the transfer of up to three embryos to the surrogate and provides limited practice guidelines. Therefore, there is little medical advice to guide to clinicians who help to produce more than 25,000 children who are now thought to be born [16]. The authorities in

Thailand see medical tourism as an opportunity for their health system, since this demands from the health services better health quality environments and integrated development as well as novel medical therapeutics [17].

On the other hand, the fact that both countries have many centers of surrogacy brings again people from the Ethics and Health Authorities to condemn the "traditional stratified world" rather than to have in this era of globalization a "flat world" [18]. The seeking by patients in high-income nations of surrogate mothers in low-income nations, particularly India, presents a set of largely unexamined ethical challenges [19].

12.8 Best Practice Guidelines for Cross-Border Reproductive Care

So far, ESHRE is the only medical society that provides clear guidance for centers and physicians providing fertility treatment to foreign patients [20, 21]. This guide aims to ensure high-quality and safe-assisted reproduction treatment, taking into account the patients, their future child, and the interests of third-party collaborators such as gametes donors and surrogates. This is achieved by including considerations of equity, safety, efficiency, effectiveness (including evidence-based care), timeliness, and patient centeredness. ESHRE deals with the ethical principles of CBRC, which are mentioned in the beginning of this chapter. Likewise, it deals with the consequences of CBRC and the professional responsibilities. ESHRE mentions the risk of exploitation of vulnerable females in the population of poor countries, especially when dealing with egg donors and surrogate mothers. Another consequence can also be the increase of fees of the treatments to the moment that these treatments will become inaccessible to local patients of those countries.

Side by side, ESHRE expresses the responsibility of the physicians to supply the full information and make sure that the standard of treatment is good. ESHRE Task Force also mentions that fee splitting is unacceptable to prevent referrals for financial reasons.

12.9 Summary

CBRC cannot be stopped. With the globalization and the easy accessibility, this phenomenon will only increase. There is a clear correlation between legal prohibitions in patient's country of origin and the number of patients who travel abroad. Therefore, societies and lawmakers should meet from time to time and examine whether old restrictions in their own countries should still be in power, or new views and attitudes can implement new and more liberal legislations in order to reduce the intensity of reproductive tourism from their countries.

These issues have to be handled in full transparency and only legally, preferably following open discussions in ethical committees and parliaments. A system of certification may be introduced to guarantee safety and effectiveness of treatment. Health systems in the countries of origin and countries of the egg donors and surrogate mothers should control the CBRC and follow them in national database systems. In this manner, the patients using the CBRC and the donors and surrogates will feel safe and protected together with good standard of treatment, which will be provided by the medical centers.

References

1. Pennings G. Legal harmonization and reproductive tourism in Europe. Hum Reprod. 2004;19(12):2689–94.
2. Pennings G, de Wert G, Shenfield F, et al. ESHRE task force on ethics and law 15: cross-border reproductive care. Hum Reprod. 2008;23(10):2182–4.
3. Pennings G. Reproductive tourism as moral pluralism in motion. J Med Ethics. 2002;28(6):337–41.
4. Matorras R. Reproductive exile versus reproductive tourism. Hum Reprod. 2005;20(12):3571; author reply -2.
5. Inhorn MC, Patrizio P. Rethinking reproductive "tourism" as reproductive "exile". Fertil Steril. 2009;92(3):904–6.
6. Matorras R. Reply: reproductive exile versus reproductive tourism. Hum Reprod. 2005;20(12):3571.
7. Follow-up to the highlevel reflection process on patient mobility and healthcare developments in the European Union. Brussels; 2004.
8. Rose H, Rose S. Playing God. The Guardian. 2003.

9. Pennings G, Autin C, Decleer W, et al. Cross-border reproductive care in Belgium. Hum Reprod. 2009;24(12):3108–18.
10. Collins J, Cook J. Cross-border reproductive care: now and into the future. Fertil Steril. 2010;94(1):e25–6.
11. College of Physicians "Reproductive Medicine" and Belgain Register for Assisted Procreation (2001) Verslag 1998–1999.
12. McKelvey A, David AL, Shenfield F, et al. The impact of cross-border reproductive care or 'fertility tourism' on NHS maternity services. BJOG. 2009;116(11):1520–3.
13. Parliament E. Resolution on the trade in human egg cells. Texts adopted, Thursday, 10 Mar 2005. Strasbourg; 2005.
14. HFEA. Re-register as an identifiable donor. 2012 [cited 2013 Feb 11]. Available from http://www.hfea.gov.uk/1973.html.
15. Eggs Donation Law. Sefer HaChukkim. 2010; 5770(2242):520.
16. Shetty P. India's unregulated surrogacy industry. Lancet. 2012;380(9854):1633–4.
17. James PT. The impact of medical tourism on Thai private hospital management: informing hospital policy. Glob J Health Sci. 2012;4(1):127–39.
18. Franklin S. Not a flat world: the future of cross-border reproductive care. Reprod Biomed Online. 2011; 23(7):814–6.
19. Deonandan R, Green S, van Beinum A. Ethical concerns for maternal surrogacy and reproductive tourism. J Med Ethics. 2012;38(12):742–5.
20. Shenfield F, Pennings G, De Mouzon J, et al. ESHRE's good practice guide for cross-border reproductive care for centers and practitioners. Hum Reprod. 2011;26(7):1625–7.
21. Shenfield F. Implementing a good practice guide for CBRC: perspectives from the ESHRE Cross-Border Reproductive Care Taskforce. Reprod Biomed Online. 2011;23(5):657–64.

Part IV

Evolving Controversies in Contemporary Reproductive Medicine

Intrauterine Insemination: An Ineffective Treatment

13

Erica B. Johnstone and Jessie Dorais

13.1 Background

The first paper on intrauterine insemination (IUI) was published by Cohen in the International Journal of Fertility in 1962 [1]. Twenty-five years later, ovarian stimulation and timed IUI was proposed by Dodson et al. for patients with unexplained infertility that had failed other treatment modalities, as a potential alternative to gamete intrafallopian transfer or in vitro fertilization [2]. These authors hypothesized the likelihood of conception would increase by increasing the number of gametes at the site of fertilization [2]. As typically performed, the IUI procedure involves removing the seminal plasma from the ejaculated semen specimen to avoid prostaglandin induced uterine contractions and pelvic infection, concentrating the sperm in culture media to promote capacitation and the acrosome reaction, and finally, dispensing the concentrated sperm into the uterine cavity using a small catheter near the time of ovulation [3]. Since its introduction over 50 years ago, IUI has evolved with changes in sperm preparation and the additions of cycle monitoring and induced ovulation with human chorionic gonadotropin in ovarian stimulation cycles.

E.B. Johnstone, M.D., M.H.S. (✉) • J. Dorais, M.D.
Reproductive Endocrinology and Infertility,
Utah Center for Reproductive Medicine,
University of Utah, Salt Lake City, UT, USA
e-mail: Erica.johnstone@hsc.utah.edu

Despite limited evidence of success for any indication, the IUI procedure is commonly utilized in unexplained infertility, mild male factor infertility, minimal-to-mild endometriosis, or as an empirical treatment for a broad range of pro-fertility indications [4]. Because the treatment premise of the IUI procedure is based on increasing the number of gametes at the site of fertilization, most IUI cycles are performed in conjunction with ovulation induction or ovarian hyperstimulation, which are associated with a significant risk of multifetal gestations, which is not effectively controlled by stimulation monitoring. Further, the success of the IUI procedure has remained weak and stagnant, whereas success rates in IVF continue to improve. The discrepancy between successful reproductive outcomes and the risk associated with multifetal gestations will continue to grow between stimulated IUI and IVF as the success rates in IVF continue to improve, particularly as patients and providers continue to increase the utilization of elective single embryo transfer. Finally, the cost analysis data on immediate IVF versus IUI followed by IVF disfavors the initial utilization of unstimulated or stimulated IUI as a cost-effective treatment modality for patients with male factor or unexplained infertility. Herein, we present data to support the argument that IUI should no longer be a standard part of infertility treatment, based on a lack of evidence supporting its efficacy, the risk of adverse events, and cost considerations.

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_13,

13.2 IUI Versus Intercourse

Many trials evaluating the efficacy of IUI utilize control populations that undergo timed intercourse (TIC) instead of ordinary intercourse, which may falsely inflate the reported therapeutic benefit of IUI. Timed intercourse dictates that couples abstain from natural coital practices for a period of time prior to the detection of an LH surge, which may reduce the likelihood of pregnancy [4]. This theory is supported by several studies that suggest that the practice of timing the IUI procedure according to the LH surge is appropriate; however, such timing might allow the optimal period for conception via intercourse to pass [4–6]. One study noted that among 221 healthy women attempting conception over 625 menstrual cycles, all recorded pregnancies were associated with intercourse during a 6-day period ending on the day of ovulation (Fig. 13.1) [5]. These authors concluded that chances of conception decline soon after ovulation and that couples abstaining from intercourse until the documentation of the LH surge may miss earlier opportunities for conception [5]. For this reason, we propose that ordinary intercourse, or expectant management, is a more appropriate control in studies of the relative efficacy of IUI. Studies utilizing TIC likely inflate the benefit of IUI and should be interpreted with caution (Fig. 13.1).

13.3 Unstimulated IUI

13.3.1 Cervical Factor Infertility

IUI has been proposed as a specific treatment for cervical hostility, or cervical factor infertility. Although small studies have suggested a benefit for IUI over expectant management in this diagnosis, a statistically significant improvement in ongoing pregnancy rates was not demonstrated [7]. Further, the utility of the postcoital test in defining this phenomenon has been strongly questioned, and a systematic review of five randomized controlled trials found no evidence of efficacy for IUI for this indication [8].

13.3.2 IUI in Male Factor Infertility

IUI has also been suggested as a treatment to overcome male factor infertility as well as to alleviate infertility associated with antisperm antibodies [9]. However, it has been shown that the intrauterine placement of prepared spermatozoa does not alter the frequency of the production of antisperm antibodies in patients undergoing IUI, and it is thus unlikely to treat or prevent infertility associated with this condition [10]. Further, a review that included outcomes for 5,214 IUI cycles from 22 trials concluded that the odds ratio

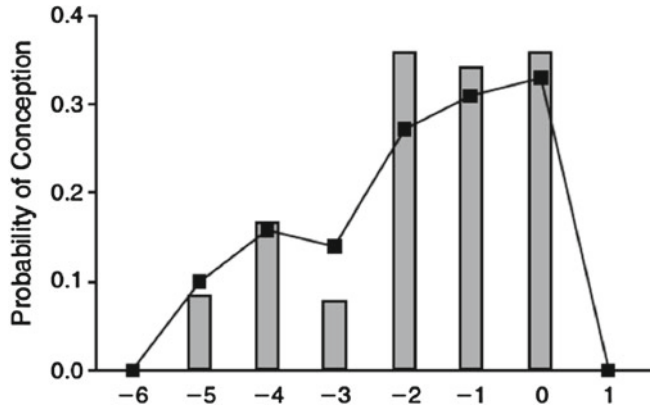

Fig. 13.1 Probability of conception on specific days near the day of ovulation. The *bars* represent probabilities calculated from data on 129 menstrual cycles in which sexual intercourse was recorded to have occurred on only a single day during the 6-day interval ending on the day of ovulation (Day 0). The *solid line* shows daily probabilities based on all 625 cycles, as estimated by the statistical model (Reprinted with permission from Wilcox et al. [5])

for pregnancy was 0.48 [95 % confidence interval (CI), 0.37–0.61] when IUI was performed for male factor, compared to all other diagnoses [11]. Another meta-analysis included data from randomized control trials to assess the efficacy of IUI for male subfertility [12]. These authors reported there was no statistically significant difference when comparing pregnancy rates in IUI versus TIC in natural cycles for male subfertility ($n=21$, OR 5.3, 95 % CI 0.42–67) [12]. The authors concluded that for male subfertility, there was insufficient evidence from randomized control trials to demonstrate improved live birth rates or ongoing pregnancy rates compared to TIC [12]. Since publication of these, an additional crossover study failed to demonstrate a benefit for IUI in natural cycles over TIC in male factor infertility [13].

13.3.3 Unexplained Infertility

IUI has also been proposed as an empiric therapy for unexplained subfertility. However, multiple studies have demonstrated no benefit for this therapy over expectant management. Bhattacharya et al. randomized 580 women with 2 years of unexplained infertility to expectant management, oral CC, or unstimulated IUI for 6 months [14]. They found that compared with expectant management, the odds ratio for a live birth was 1.46 (0.88–2.43) after unstimulated IUI, which was not statistically significant despite a large sample size [14]. Thus, when utilized for male factor or unexplained infertility, the utilization of unstimulated IUI for unexplained infertility or male factor infertility is not currently supported by the literature.

13.4 IUI with Ovarian Stimulation Versus Stimulation Alone

13.4.1 Male Factor Infertility

Data supporting an enhanced pregnancy rate when IUI is added to ovarian stimulation or superovulation is also limited. While a few studies have suggested benefit [15], this has not been supported in a recently published large meta-analysis. Bensdorp et al. evaluated the effectiveness of IUI versus TIC in stimulated cycles for couples with male subfertility, incorporating studies with varied stimulation regimens [12]. The authors found no significant improvement in pregnancy rates for stimulated cycles with IUI versus stimulated cycles with TIC for couples with male subfertility ($n=202$, OR 1.67, 95 % CI 0.83–3.37) [12].

13.4.2 Unexplained Infertility

Doubt about the effectiveness of IUI in unexplained infertility was raised many years ago and persists. Individual studies have been inconsistent on whether pregnancy rates are increased when IUI is added to COH [16–20]. Two early meta-analyses demonstrated a marginal benefit for IUI over TIC combined with COH with injectable gonadotropins for couples with unexplained infertility. Zeyneloglu et al. reported an OR for pregnancy of 1.84 (95 % CI = 1.30–2.62) among 980 cycles when IUI with FSH was compared to FSH alone [21]. Hughes reported an OR of 2.37 [95 % CI, 1.43, 3.90] for the same comparison, although they noted significant clinical heterogeneity among the 8 included trials [11]. Another study demonstrated a benefit, but the per-cycle pregnancy rate in the clomiphene citrate (CC) + IUI cohort was very low, at 3.16 %, a rate that is likely of limited acceptability to most couples [22].

Despite the aforementioned, limited number of studies documenting a small benefit for IUI for couples with unexplained infertility, these findings are not reproducible and multiple studies refute these findings. A recent meta-analysis consisting of seven trials comparing TIC with IUI in couples with unexplained infertility found no benefit for IUI [23]. Further, two recent randomized control trials also failed to demonstrate the benefit of IUI with ovarian hyperstimulation over TIC for couples with unexplained infertility. In the first study, 140 couples with unexplained infertility were randomly assigned to controlled

ovarian hyperstimulation (COH) with CC and either TIC or IUI [24]. There was no statistically significant difference in the pregnancy rate for the COH/TIC cohort (41 %) and COH/IUI (18 %) cohort over up to six cycles [24]. Another study of 157 couples with unexplained infertility randomized patients to compare outcomes of IUI, direct intraperitoneal insemination, and intercourse in cycles stimulated with CC or gonadotropins [16]. The results demonstrated that insemination cycles and intercourse cycles had a similar overall pregnancy rates of 12 % and 13 %, respectively, and the authors concluded that insemination had no beneficial effect on the pregnancy rates in stimulated cycles for treatment of unexplained infertility [16]. COH/IUI treatment has also been compared to expectant management in a study of 253 couples with unexplained infertility randomized to 6 months of IUI with controlled ovarian hyperstimulation versus 6 months of expectant management [25]. These investigators found that the conception rates of 33 % versus 32 % and ongoing pregnancy rates 23 % versus 27 % were not significantly different between the intervention group and the expectant management group, respectively (relative risk 0.85, 95 % CI 0.63–1.1), but the only triplet pregnancy was in the COH/IUI group [25]. Similarly, IUI does not increase clinical pregnancy or live birth rates for anovulatory women treated with CC with IUI versus TIC, with live birth rates per cycle 8.5 % with IUI and 7.9 % with TIC [26]. The failure to consistently demonstrate a benefit of IUI added to superovulation for unexplained infertility raises doubt that IUI offers any increase in the chances of successful pregnancy.

13.5 Cost-Effectiveness

Cost must also be considered when considering treatment strategies for infertility patients. Treatment costs associated with expectant management, oral CC, or unstimulated IUI were collected prospectively by Bhattacharya et al. [14, 27]. The cost analysis revealed the costs per live birth were £72 (95 % confidence interval £0–£206), £2611 (£1870–£4166), and £1487 (£1116–£2155) for expectant management, CC, and IUI, respectively. This led to an incremental increase in cost per additional live birth of £5604 with IUI, compared with expectant management, as depicted in Table 13.1 [14]. The authors concluded that empiric treatment with IUI for unexplained infertility was not associated with increased live birth rates and was unlikely to be a cost-effective treatment [27]. Custers et al. noted similar results in longitudinal assessment of the 253 couples with unexplained subfertility, initially randomized to expectant management or treatment with controlled ovarian stimulation IUI (COS-IUI) for 6 months [28]. After 3 years of follow-up, there was no difference between the groups in chances of pregnancy or time interval to pregnancy, but the COS-IUI group incurred an additional 2616 € in costs [28].

13.6 Adverse Events

In addition to an absence of consistent evidence supporting the efficacy and cost-effectiveness of IUI for various indications, one must also consider the risks and adverse effects associated with the IUI procedure. The adverse effects associated with the procedure include the discomfort of the procedure and the potential risk of infection. The risk of an infectious complication in women undergoing IUI has been reported to be 1.83 per 1,000 women undergoing the IUI procedure [29]. While IUI has not been shown to increase the rate of multifetal gestations, IUI is often performed in conjunction with superovulation or COH, which increases the risk of multifetal gestation far above that associated with natural conception cycles. An absence of registry information about non-ART treatments makes it difficult to analyze the contribution of ovarian stimulation plus IUI or ovulation induction plus IUI to multiple birth rates. A recent review reported the multiple pregnancy rates after non-ART ovarian hyperstimulation ranged from 10 % to 40 % per cycle and estimated the contribution of this treatment to the multiple birth epidemic to be approximately 30 % [30]. The authors noted the contribution of ovarian stimulation, with either ovulation induction

Table 13.1 Cost and effectiveness results

Treatment	Mean (SD) cost per treatment cycle[a] (£)	Mean (SD) number of treatment cycles per patient	Mean (SD) treatment cost per patient[b] (£)	Treatment cost difference versus EM (SE)	95 % CI for cost difference versus EM (*P* value) (£)	Live birth rate (per woman)	Odds ratio versus EM (95 % CI; *P* value)	Mean cost per live birth (95 % CI)[c] (£)
EM	0	–	11.88 (116.50)	–	–	0.17	–	71.64 (−27.02 to 191.51)
CC	83.12 (17.21)	4.10 (2.22)	349.96 (219.54)	338.08 (17.85)	303.39–370.02 (<0.0001)	0.13	0.78 (0.44–1.36; 0.3824)	2611.25 (1870.49 to 4166.46)
IUI	97.61 (31.12)	3.39 (2.01)	331.27 (222.15)	319.39 (18.06)	286.19–352.89 (<0.0001)	0.22	1.44 (0.87–2.40; 0.1584)	1486.87 (1116.48 to 2155.12)

[a]Weighted average, based on the total cost of treatment and the total number of treatment cycles provided in each center
[b]Incorporating the cost of adverse events
[c]Mean cost per live birth = Mean treatment cost per patient divided by live birth rate per woman (e.g., for EM: 11.88/0.17 = 71.64) (Reprinted with permission [27])

or superovulation, to triplet or higher-order multiple birth approaches 50 % [30].

In the USA between 1997 and 2000, ovarian stimulation and ovulation induction's contribution to the national multiple births increased from 18.9 % (20,955 infants) to 21.9 % (27,647 infants) [2]. The risk varies depending on the ovulation induction agent and dose. The estimated risk of multifetal gestation after treatment with CC and IUI is 8–10 % [31]. Rates of multiple gestations after gonadotropin stimulation with IUI are undoubtedly dependent upon individual clinical practices with regard to monitoring and cancellation of cycles; however, rates of twin and high-order multiples as high as 28.6 % and 8.2 %, respectively, have been reported [2]. Table 13.2 summarizes rates of multiple gestations reported with gonadotropin stimulation in a variety of studies [32].

The importance of these associated risks should not be underemphasized, as multifetal gestations are associated with significant risk to maternal, fetal, and neonatal health. Multifetal gestations carry increased risk of maternal complications including anemia, gestational diabetes, cesarean section, preeclampsia, postpartum hemorrhage, and mortality [30]. Adverse fetal and neonatal effects of multifetal gestations include infection, bleeding, prematurity, cerebral palsy, visual and hearing defects, and learning difficulties [30].

13.7 IUI Versus IVF

The effectiveness of IUI must be considered in comparison to in vitro fertilization (IVF), as multifetal gestations can be effectively prevented with IVF with elective single embryo transfer. Past studies comparing IUI and IVF become quickly dated as IUI success rates have remained stagnant, whereas IVF outcomes have continued to improve [4]. In a study published in 2000, Goverde et al. found similar per cycle and cumulative pregnancy rates with IVF, IUI, and COH/IUI and increased costs per live birth with IVF. However, the pregnancy rate per cycle in IVF was only 12.2 % [49]. In the USA, in 2010, the chances of live birth in an in vitro fertilization cycle were 41.7 % per initiated cycle and 47.8 % per embryo transfer for women under the age of 35 (SART 2010 National Data Summary). In the FASTT trial, women ages 21–39 with unexplained infertility were randomized to undergo three cycles of CC/IUI followed by three cycles of FSH/IUI, followed by IVF, or, to an accelerated track consisting of three cycles of CC/IUI followed by IVF. The investigators demonstrated not only increased pregnancy rates in the accelerated track but also a cost savings of $2624 per couple [50]. In data presented in abstract, the FORT-T Trial, by the same investigators demonstrated an increased live birth rate among women aged 38–43, undergoing immediate IVF compared with IUI preceded by either FSH or CC superovulation, with rates of 15.3 % and 5.1 %, respectively [51]. Thus, the use of COH-IUI appears to offer little benefit to patients, while increasing total costs and delaying the time to pregnancy.

Moreover, IVF with elective single embryo transfer (eSET) has been demonstrated to minimize the risks of multiple gestation associated with COH-IUI. In a recent randomized control trial evaluating outcomes after elective single embryo transfer (eSET) versus double embryo transfer (DET), no difference was demonstrated in the ongoing pregnancy rates for 61 % for eSET versus 76 % for DET (RR 0.80; p = NS), with twin rates of 47 % after DET and 0 % after eSET [52]. In another study, a single cycle of IVF with eSET was compared with three cycles of COH-IUI. Ongoing pregnancy rates were similar in the two arms, but there were no higher order multiples in the IVF group [53]. These studies clearly demonstrate the efficacy of IVF with eSET. There has been a gradual increase in the utilization of elective single embryo transfer in IVF over time worldwide [54]. This change in practice worldwide will likely continue to decrease multifetal gestations associated with IVF; however, similar options are not available to decrease multifetal gestations associated with COH-IUI. The disparity in multifetal gestations after COH-IUI versus IVF cycles will likely widen in the future as patient and provider acceptance of elective single embryo transfer continues to increase in IVF.

Table 13.2 Studies assessing multiple gestation risk associated with non-ART ovulation stimulation with gonadotropin medications or both gonadotropin and clomiphene or other anties trogenenic medications[a]

Author, year [reference number]	Study type	Time period	No. of women included	No. of total treatments	No. of pregnancies	Twin pregnancies		Triplet/+ pregnancies		Total multiple pregnancies	
						No.	%	No.	%	No.	%
Bedaiwy et al., 2007 [33]	Obs/P	2003–2006	389	630	94	16	17.0	3	3.2	19	20.2
Ragni et al., 2006 [34]	Obs/R	2001–2004	621	1,259	116	11	9.5	0	0	11	9.5
Matorras et al., 2006 [35]	Obs/R	2002–2003	NS	328	54	9	16.7	2	3.7	11	20.4
Gorry et al., 2006 [36]	Obs/R	1990–2002	199	916	91	3	3.3	0	0	3	3.3
Tur et al., 2005 [37]	Obs/P	2001–2002	NS	1,542	207	33	15.9	5	2.4	38	18.4
Mitwally et al., 2005 [38][b]	Obs/P	1999–2001	NS	671	95	NS	~12	0	0	NS	~12
Mitwally et al., 2005 [38][b]	Obs/P	1999–2001	NS	358	57	NS	~13	0	0	NS	~13
Dickey et al., 2005 [39]	Obs/R	1987–2002	2,272	4,067	587	108	18.4	38	6.5	146	24.9
Ibérico et al., 2001 [40]	Obs/R	2000–2002	470	1,010	93	NS	8.6	0	0	NS	8.6
Calaf Alsina et al., 2003 [41]	Obs/P	1988–1999	343	945	136	8	5.9	0	0	8	5.9
Tur et al., 2001 [42]	Obs/R	1988–1998	NS	NS	1,878	294	15.7	107	5.7	401	21.4
Schachter et al., 2001 [43]	Obs/R	1997–1999	220	480	129	11	8.5	3	2.3	14	10.9
Gleicher et al., 2000 [44]	Obs/R	1997–1998	1,494	3,347	441	88	20.0	39	8.8	127	28.8
Nuojua-Huttunen et al., 1999 [45]	Obs/R	1992–1996	NS	811	102	12	11.8	2	2.0	14	13.7
Ragni et al., 1999 [46]	NS	NS	273	449	51	12	23.5	1	2.0	13	25.5
De Geyter et al., 1998 [47]	Obs/R	1989–1996	410	796	163	15	9.2	5	3.1	20	12.3
Tadokoro et al., 1997 [48]	Obs/R	1991–1995	356	995	187	26	13.9	5	2.7	31	16.6
Posterior mean						12.32		1.99			
95% Credible interval						9.05	16.16	0.90	3.54		

ART assisted reproductive technology, *NS* not stated, *Obs/P* observational, prospective, *Obs/R* observational, retrospective, *RT* randomized trial, *triplet/+* triplet or higher order

[a]From each study, data for each non-ART ovulation treatment group that include ≥50 pregnancies were abstracted separately. Thus, some studies contributed more than one treatment group to summary calculations

[b]Exact numbers on twin pregnancies were not provided, but twinning rates were estimated from bar graphs. Triplet/+ pregnancies were stated as 0

(Reprinted with permission [32])

13.8 Cost-Effectiveness of IVF Versus IUI

Despite the greater cost per cycle of IVF compared with COH-IUI, cost-effectiveness data favors immediate IVF. Pashayan et al. used mathematical modeling to estimate the cost-effectiveness of first-line treatment with IVF (including cryopreservation cycles) versus initial treatment with either stimulated or unstimulated IUI followed by IVF for couples who did not become pregnant with IUI on 100 theoretical patients with male factor or unexplained infertility [55]. The authors concluded that for this hypothetical cohort of 100 couples, compared with an initial offer of IVF, six cycles of unstimulated IUI followed by IVF would cost an additional £174,200 and stimulated IUI followed by IVF would cost an additional £438,000 [55]. They also reported this cost in terms of the opportunity cost. The authors reported the opportunity cost for initiating treatment with unstimulated IUI followed by IVF was 54 IVF cycles and 14 live births and the opportunity cost of stimulated IUI followed by IVF was 136 IVF cycles and 35 live births for that health care system [55]. Although an individual may experience a cost saving if she were to become pregnant with stimulated or unstimulated IUI, these studies reveal an overall cost savings per live birth for a population of couples with male factor or unexplained infertility. Modeling from this study is depicted in Fig. 13.2.

IVF is widely accepted as preferred therapy for bilateral tubal obstruction, and severe oligozoospermia, where chances of conception with IUI are extremely low. In addition to a lack of evidence from randomized control trials supporting the utilization of IUI in male subfertility, there are inconsistent thresholds below which IUI would be an ineffective treatment option [4, 56, 57]. One retrospective study of more than 1,800 patients concluded that pregnancy rates were at least 8.2 % when initial sperm values

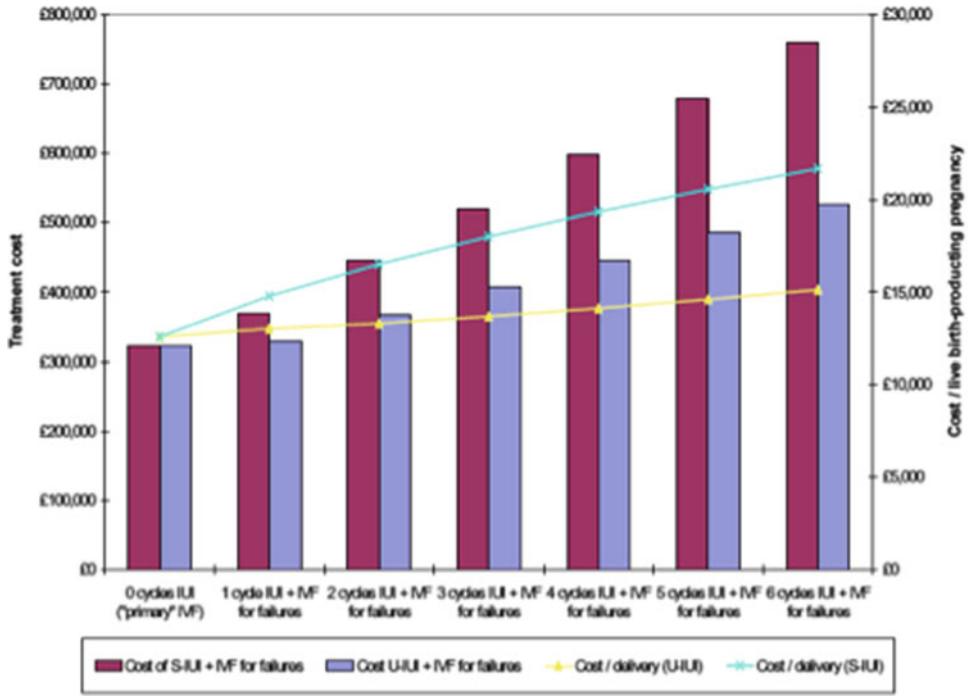

Fig. 13.2 Cost and cost-effectiveness (per live birth-producing pregnancy) of different uptake of IUI and S-IUI among a hypothetical cohort of 100 couples eligible for both IUI and IVF. Assume constant LBR of 7 % and 3.5 % for S-IUI and IUI (Reprinted with permission from Pashayan et al. [39])

demonstrated greater than or equal to a concentration of two million per mL, a total count of ten million, progressive motility of 30 %, and a total motile sperm count of five million [58]. These authors reported pregnancy rates less than 3.6 % when initial sperm values were below these thresholds, but above the lowest initial sperm values associated with a pregnancy: a concentration of two million per mL, a total count of five million, motility of 17 %, and a total motile sperm count of 1.6 million [58].

A second retrospective study of over 2,400 IUI cycles reported pregnancy rates of 5.3 % if the semen analysis demonstrated less than five million motile sperm versus 12.8 % with samples greater than five million motile sperm ($p < 0.02$) [57]. A third retrospective study looked at the relative effectiveness and cost-effectiveness based on sperm counts in 3,479 IUI cycles and 551 IVF cycles [56]. These investigators concluded that when the average total motile sperm count was under ten million, IVF with ICSI was more cost-effective than IUI, and proposed that an average total motile sperm count of less than ten million be used as a threshold for recommending IVF with ICSI over IUI [56]. These discrepant thresholds further complicate the decision making for patients and providers considering treatment options in cases of male factor subfertility. Regardless of the ideal threshold for recommending IVF over IUI in cases of male factor subfertility, the fact remains there is an absence of clear data from well-designed randomized studies supporting the utilization of IUI in cases of male factor infertility [12].

13.9 Conclusion

Current evidence fails to support the continued utilization of IUI for male factor or unexplained infertility. The IUI procedure is often performed in conjunction with ovulation induction or controlled ovarian stimulation, which is associated with an inherent, excessive, and unavoidable risk of producing a multifetal gestation. Further, despite the chance that an individual may experience a cost saving if a pregnancy were achieved after COH/IUI, studies considering a population of infertile patients do not support the utilization of IUI as a cost-effective treatment. Thus, based on a lack of data demonstrating efficacy, cost considerations, and the adverse effects associated with the procedure as it is typically performed, IUI should no longer be offered as part of routine treatment in modern day infertility practices.

References

1. Cohen MR. Intrauterine insemination. Int J Fertil. 1962;7:235–40.
2. Dodson WC, Whitesides DB, Hughes CL, Jr., Easley HA, 3rd, Haney AF. Superovulation with intrauterine insemination in the treatment of infertility: a possible alternative to gamete intrafallopian transfer and in vitro fertilization. Fertil Steril. 1987;48(3):441–5.
3. Yen SSC, Strauss JF, Barbieri RL. Yen and Jaffe's reproductive endocrinology: physiology, pathophysiology, and clinical management. 5th ed. Philadelphia, PA: Elsevier/Saunders; 2004.
4. ESHRE Capri Workshop Group. Intrauterine insemination. Hum Reprod update. 2009;15(3):265–77.
5. Wilcox AJ, Weinberg CR, Baird DD. Timing of sexual intercourse in relation to ovulation. Effects on the probability of conception, survival of the pregnancy, and sex of the baby. N Engl J Med. 1995;333(23):1517–21.
6. Nulsen J, Wheeler C, Ausmanas M, et al. Cervical mucus changes in relationship to urinary luteinizing hormone. Fertil Steril. 1987;48(5):783–6.
7. Steures P, van der Steeg JW, Hompes PG, et al. Effectiveness of intrauterine insemination in subfertile couples with an isolated cervical factor: a randomized clinical trial. Fertil Steril. 2007;88(6):1692–6.
8. Helmerhorst FM, van Vliet HA, Gornas T, et al. Intrauterine insemination versus timed intercourse for cervical hostility in subfertile couples. Obstet Gynecol Surv. 2006;61(6):402–14; quiz 23.
9. Robinson JN, Forman RG, Nicholson SC, et al. A comparison of intrauterine insemination in superovulated cycles to intercourse in couples where the male is receiving steroids for the treatment of autoimmune infertility. Fertil Steril. 1995;63(6):1260–6.
10. Horvath PM, Beck M, Bohrer MK, et al. A prospective study on the lack of development of antisperm antibodies in women undergoing intrauterine insemination. Am J Obstet Gynecol. 1989;160(3):631–7.
11. Hughes EG. The effectiveness of ovulation induction and intrauterine insemination in the treatment of persistent infertility: a meta-analysis. Hum Reprod. 1997;12(9):1865–72.
12. Bensdorp AJ, Cohlen BJ, Heineman MJ, et al. Intrauterine insemination for male subfertility. Cochrane Database Syst Rev. 2007;(3):CD000360.

13. Francavilla F, Sciarretta F, Sorgentone S, et al. Intrauterine insemination with or without mild ovarian stimulation in couples with male subfertility due to oligo/astheno- and/or teratozoospermia or antisperm antibodies: a prospective cross-over trial. Fertil Steril. 2009;92(3):1009–11.
14. Bhattacharya S, Harrild K, Mollison J, et al. Clomifene citrate or unstimulated intrauterine insemination compared with expectant management for unexplained infertility: pragmatic randomised controlled trial. Br Med J. 2008;337:a716.
15. Gregoriou O, Vitoratos N, Papadias C, et al. Pregnancy rates in gonadotrophin stimulated cycles with timed intercourse or intrauterine insemination for the treatment of male subfertility. Eur J Obstet Gynecol Reprod Biol. 1996;64(2):213–6.
16. Karlstrom PO, Bergh T, Lundkvist O. A prospective randomized trial of artificial insemination versus intercourse in cycles stimulated with human menopausal gonadotropin or clomiphene citrate. Fertil Steril. 1993;59(3):554–9.
17. Zikopoulos K, West CP, Thong PW, et al. Homologous intra-uterine insemination has no advantage over timed natural intercourse when used in combination with ovulation induction for the treatment of unexplained infertility. Hum Reprod. 1993;8(4):563–7.
18. Gregoriou O, Vitoratos N, Papadias C, et al. Controlled ovarian hyperstimulation with or without intrauterine insemination for the treatment of unexplained infertility. Int J Gynaecol Obstet. 1995;48(1):55–9.
19. Arcaini L, Bianchi S, Baglioni A, et al. Superovulation and intrauterine insemination vs. superovulation alone in the treatment of unexplained infertility. A randomized study. J Reprod Med. 1996;41(8):614–8.
20. Melis GB, Paoletti AM, Ajossa S, et al. Ovulation induction with gonadotropins as sole treatment in infertile couples with open tubes: a randomized prospective comparison between intrauterine insemination and timed vaginal intercourse. Fertil Steril. 1995; 64(6):1088–93.
21. Zeyneloglu HB, Arici A, Olive DL, et al. Comparison of intrauterine insemination with timed intercourse in superovulated cycles with gonadotropins: a meta-analysis. Fertil Steril. 1998;69(3):486–91.
22. Aribarg A, Sukcharoen N. Intrauterine insemination of washed spermatozoa for treatment of oligozoospermia. Int J Androl. 1995;18 Suppl 1:62–6.
23. Ford WC, Mathur RS, Hull MG. Intrauterine insemination: is it an effective treatment for male factor infertility? Baillieres Clin Obstet Gynaecol. 1997; 11(4):691–710.
24. Agarwal S, Mittal S. A randomised prospective trial of intrauterine insemination versus timed intercourse in superovulated cycles with clomiphene. Indian J Med Res. 2004;120(6):519–22.
25. Steures P, van der Steeg JW, Hompes PG, et al. Intrauterine insemination with controlled ovarian hyperstimulation versus expectant management for couples with unexplained subfertility and an intermediate prognosis: a randomised clinical trial. Lancet. 2006;368(9531):216–21.
26. Abu Hashim H, Ombar O, Abd Elaal I. Intrauterine insemination versus timed intercourse with clomiphene citrate in polycystic ovary syndrome: a randomized controlled trial. Acta Obstet Gynecol Scand. 2011;90(4):344–50.
27. Wordsworth S, Buchanan J, Mollison J, et al. Clomifene citrate and intrauterine insemination as first-line treatments for unexplained infertility: are they cost-effective? Hum Reprod. 2011;26(2): 369–75.
28. Custers IM, van Rumste MM, van der Steeg JW, et al. Long-term outcome in couples with unexplained subfertility and an intermediate prognosis initially randomized between expectant management and immediate treatment. Hum Reprod. 2012;27(2): 444–50.
29. Sacks PC, Simon JA. Infectious complications of intrauterine insemination: a case report and literature review. Int J Fertil. 1991;36(6):331–9.
30. Fauser BC, Devroey P, Macklon NS. Multiple birth resulting from ovarian stimulation for subfertility treatment. Lancet. 2005;365(9473):1807–16.
31. Tarlatzis BC, Grimbizis G. Future use of clomiphene in ovarian stimulation. Will clomiphene persist in the 21st century? Hum Reprod. 1998;13(9):2356–8.
32. Schieve LA, Devine O, Boyle CA, et al. Estimation of the contribution of non-assisted reproductive technology ovulation stimulation fertility treatments to US singleton and multiple births. Am J Epidemiol. 2009;170(11):1396–407.
33. Bedaiwy MA, Mousa NA, Esfandiari N, Forman R, Casper RF. Follicular phase dynamics with combined aromatase inhibitor and follicle stimulating hormone treatment. J Clin Endocrinol Metab. 2007;92(3): 825–33.
34. Ragni G, Caliari I, Nicolosi AE, Arnoldi M, Somigliana E, Crosignani PG. Preventing high-order multiple pregnancies during controlled ovarian hyperstimulation and intrauterine insemination: 3 years' experience using low-dose recombinant follicle-stimulating hormone and gonadotropin-releasing hormone antagonists. Fertil Steril. 2006;85(3):619–24.
35. Matorras R, Ramon O, Exposito A, Corcostegui B, Ocerin I, Gonzalez-Lopera S, et al. Gn-Rh antagonists in intrauterine insemination: the weekend-free protocol. J Assist Reprod Genet. 2006;23(2):51–4.
36. Gorry A, White DM, Franks S. Infertility in polycystic ovary syndrome: focus on low-dose gonadotropin treatment. Endocrine. 2006;30(1):27–33.
37. Tur R, Barri PN, Coroleu B, Buxaderas R, Parera N, Balasch J. Use of a prediction model for high-order multiple implantation after ovarian stimulation with gonadotropins. Fertil Steril. 2005;83(1):116–21.
38. Mitwally MF, Biljan MM, Casper RF. Pregnancy outcome after the use of an aromatase inhibitor for ovarian stimulation. Am J Obstet Gynecol. 2005;192(2):381–6.

39. Dickey RP, Taylor SN, Lu PY, Sartor BM, Rye PH, Pyrzak R. Risk factors for high-order multiple pregnancy and multiple birth after controlled ovarian hyperstimulation: results of 4,062 intrauterine insemination cycles. Fertil Steril. 2005;83(3):671–83.
40. Iberico G, Vioque J, Ariza N, Lozano JM, Roca M, Llacer J, et al. Analysis of factors influencing pregnancy rates in homologous intrauterine insemination. Fertil Steril. 2001;81(5):1308–13.
41. Calaf Alsina J, Ruiz Balda JA, Romeu Sarrio A, Caballero Fernandez V, Cano Trigo I, Gomez Parga JL, et al. Ovulation induction with a starting dose of 50 IU of recombinant follicle stimulating hormone in WHO group II anovulatory women: the IO-50 study, a prospective, observational, multicentre, open Trial. BJOG. 2003;110(12):1072–7.
42. Tur R, Barri PN, Coroleu B, Buxaderas R, Martinez F, Balasch J. Risk factors for high-order multiple implantation after ovarian stimulation with gonadotrophins: evidence from a large series of 1878 consecutive pregnancies in a single centre. Hum Reprod. 2001;16(10):2124–9.
43. Schachter M, Raziel A, Friedler S, Strassburger D, Bern O, Ron-El R. Monozygotic twinning after assisted reproductive techniques: a phenomenon independent of micromanipulation. Hum Reprod. 2001;16(6):1264–9.
44. Gleicher N, Oleske DM, Tur-Kaspa I, Vidali A, Karande V. Reducing the risk of high-order multiple pregnancy after ovarian stimulation with gonadotropins. N Engl J Med. 2000;343(1):2–7.
45. Nuojua-Huttunen S, Tomas C, Bloigu R, Tuomivaara L, Martikainen H. Intrauterine insemination treatment in subfertility: an analysis of factors affecting outcome. Hum Reprod. 1999;14(3):698–703.
46. Ragni G, Maggioni P, Guermandi E, Testa A, Baroni E, Colombo M, et al. Efficacy of double intrauterine insemination in controlled ovarian hyperstimulation cycles. Fertil Steril. 1999;72(4):619–22.
47. De Geyter C, De Geyter M, Nieschlag E. Low multiple pregnancy rates and reduced frequency of cancellation after ovulation induction with gonadotropins, if eventual supernumerary follicles are aspirated to prevent polyovulation. J Assist Reprod Genet. 1998;15(3):111–6.
48. Tadokoro N, Vollenhoven B, Clark S, Baker G, Kovacs G, Burger H, et al. Cumulative pregnancy rates in couples with anovulatory infertility compared with unexplained infertility in an ovulation induction programme. Hum Reprod. 1997;12(9):1939–44.
49. Goverde AJ, McDonnell J, Vermeiden JP, et al. Intrauterine insemination or in-vitro fertilisation in idiopathic subfertility and male subfertility: a randomised trial and cost-effectiveness analysis. Lancet. 2000;355(9197):13–8.
50. Reindollar RH, Regan MM, Neumann PJ, et al. A randomized clinical trial to evaluate optimal treatment for unexplained infertility: the fast track and standard treatment (FASTT) trial. Fertil Steril. 2010;94(3):888–99.
51. Reindollar RH, Thornton KL, Ryley D. A randomized clinical trial to determine optimal infertility therapy in couples when the female partner is 38-42 years: preliminary results from the forty and over infertility treatment trial (FORT-T). Fertil Steril. 2011;96(3) Suppl:S1.
52. Gardner DK, Surrey E, Minjarez D, et al. Single blastocyst transfer: a prospective randomized trial. Fertil Steril. 2004;81(3):551–5.
53. Custers IM, Konig TE, Broekmans FJ, et al. Couples with unexplained subfertility and unfavorable prognosis: a randomized pilot trial comparing the effectiveness of in vitro fertilization with elective single embryo transfer versus intrauterine insemination with controlled ovarian stimulation. Fertil Steril. 2011;96(5):1107–11.e1.
54. Practice committee of society for assisted reproductive technology; practice committee of american society for reproductive medicine. Elective single-embryo transfer. Fertil Steril. 2012;97(4):835–42.
55. Pashayan N, Lyratzopoulos G, Mathur R. Cost-effectiveness of primary offer of IVF vs. primary offer of IUI followed by IVF (for IUI failures) in couples with unexplained or mild male factor subfertility. BMC Health Serv Res. 2006;6:80.
56. Van Voorhis BJ, Barnett M, Sparks AE, et al. Effect of the total motile sperm count on the efficacy and cost-effectiveness of intrauterine insemination and in vitro fertilization. Fertil Steril. 2001;75(4):661–8.
57. Khalil MR, Rasmussen PE, Erb K, et al. Homologous intrauterine insemination. An evaluation of prognostic factors based on a review of 2473 cycles. Acta Obstet Gynecol Scand. 2001;80(1):74–81.
58. Dickey RP, Pyrzak R, Lu PY, et al. Comparison of the sperm quality necessary for successful intrauterine insemination with World Health Organization threshold values for normal sperm. Fertil Steril. 1999;71(4):684–9.

IUI Is a Valuable and Cost-Effective Therapy for Most Couples

14

Lobke M. Moolenaar, Bradley J. Van Voorhis, and Fulco van der Veen

14.1 Introduction

To maximize population health outcomes with the minimum possible use of resources, cost-effectiveness of interventions is an important consideration in any health care system. In reproductive medicine, costs play an important role, due to the rise in healthcare costs and since infertility care is not a covered benefit in many countries. The choice for one fertility treatment over the other is frequently driven by financial considerations rather than by cost-effectiveness.

14.2 Cost-Effectiveness

In some cases, the most cost-effective treatment for couples presenting with infertility is straightforward. For women presenting with severe tubal pathology, the most cost-effective treatment is IVF, since natural conception is almost nonexisting [1]. For women with anovulation, ovulation induction is the logical treatment. But in most couples presenting with infertility, the most cost-effective treatment is not clear. In couples with mild-to-moderate male factor, mild endometriosis, cervical factor, unexplained infertility, or in case of persistent infertility in women with anovulation who had their ovulation restored, several treatment options are available.

Fertility treatment pathways generally move from low-cost, low-technology treatments such as intrauterine insemination (IUI) to high-cost, more invasive assisted reproductive technologies (ART) with in vitro fertilization (IVF) [2–4]. Although we often assume that patients want "treatment," counseling about the chances and ways to improve the chances for natural conception should be considered in the treatment pathway. A model has been developed, which accurately predicts the chances of spontaneous conception in subfertile couples by taking into account several prognostic factors including female age, parity, duration of infertility, fallopian tube status, and sperm motility [5]. This can be used to counsel patients and also to categorize couples by prognosis, which is particularly important when comparing a treatment with an expectant management arm in a study. Apart from varying costs and effectiveness between treatments, time to pregnancy must also be considered to avoid the negative effects of aging on women's reproductive potential.

L.M. Moolenaar, M.D. (✉)
Center for Reproductive Medicine,
Academic Medical Center, University of Amsterdam,
Amsterdam, The Netherlands
e-mail: l.m.moolenaar@amc.uva.nl

B.J. Van Voorhis, M.D.
Center for Advanced Reproductive Care,
University of Iowa, Iowa City, IA, USA

F. van der Veen, M.D., Ph.D.
Amsterdam Academic Medical Centre,
University of Amsterdam, Amsterdam,
North-Holland, The Netherlands

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_14,

A randomized controlled trial comparing expectant management for 6 months to hMG-IUI for 6 months in women with a mean age of 33 and a duration of infertility of 2 years and a prognosis for natural conception of 30–40 % within a year, calculated according to the model of Hunault showed no differences in pregnancy rates between the two interventions and, based only on direct costs, that immediate treatment with IUI was twice as expensive as expectant management [5, 6]. Therefore, expectant management for 6 months is first-line treatment for couples presenting with infertility and a prognosis between 30 and 40 %, which represents an intermediate prognosis. A follow-up study, in which the women were treated according to local protocol at the conclusion of the original study, showed that also after 3 years there was no difference in pregnancy rate between the women treated with IUI for 6 months and with expectant management for 6 months [7–10].

In the last two decades three prospective studies compared the cost-effectiveness of IUI in relation to IVF [8–10]. In 1999 two strategies in 96 women were compared [9]. A strategy of immediate IVF treatment was compared with a standard infertility treatment algorithm (SITA) of CC-IUI, hmG-IUI, and IVF in couples with all types of infertility. Duration of infertility was between 23 and 30 months as a mean for the two arms of the study. Thirty two percent of all pregnancies occurred without active treatment in "rest" cycles confirming the observation that couples with infertility can conceive at a reasonably high rate with no therapy at all. This study suggested IVF not to be cost-effective as a first-line treatment in couples with unexplained infertility compared with an SITA. Cost per pregnancy after IVF treatment was twice as high as treatment with SITA, US$38,021 and US$16,725, respectively. This study had several limitations. First, four sets of triplets and five sets of twins occurred, but the cost of multiple pregnancies was not included. Second, the cost analysis was performed with insurance charges, which does not necessarily reflect the true costs.

The second randomized trial was performed in 258 couples with unexplained and mild male infertility undergoing IVF, natural cycle IUI, or hMG-IUI [10]. Mean duration of infertility in the three study arms varied between 46.5 and 53.4 months. This study did not find any difference in cumulative pregnancy rates but did find a difference in cost per pregnancy. IVF was 2.5 times more expensive than IUI and hMG-IUI. IUI was also better tolerated by couples as more couples continued with this therapy, while IVF was associated with a higher drop-out rate. In this study only treatment costs were included, antenatal and postnatal costs of multiple pregnancies were not included. The multiple pregnancy rate in the hMG-IUI arm was 27 % and if included could impact the cost-effectiveness tremendously. Of note is the extremely low IVF pregnancy rate of 12.2 % per cycle, a value which is clearly outdated as compared to modern IVF standards. Nevertheless, the relative cost-effectiveness of IUI was demonstrated with little additional effect seen by adding Gonadotropin stimulation to the treatment prior to IUI.

A recent study, the Fast Track and Standard Treatment trial (FAST-trial) compared a conventional arm of three cycles of CC-IUI, three cycles of FSH-IUI, and six cycles of IVF to an accelerated arm of three cycles of CC-IUI and six cycles of IVF [8]. This study included women with unexplained subfertility. Time to conception was included in this study. The cost of multiple gestations and their associated increased hospital perinatal costs were included in this analysis. Out-of-pocket expenses (indirect costs) to the patient were also calculated. This study concluded that after initial CC-IUI moving directly to IVF results in lower cost and a shorter time to pregnancy. The median time in pregnancy differences was 3 months and there was an equivalent rate of multiple pregnancies in both treatment arms. Ultimately, by 1 year, the pregnancy rate in the two arms was equal. A limitation of that study is that the mean and median duration of unexplained subfertility was not provided and the prognostic profile of the included couples cannot be extracted from the paper. This is important in interpreting the effectiveness of IUI with superovulation versus expectant management [6, 7]. In the FAST trial 14 % of the pregnancies were treatment independent.

14.3 Discussion

Four studies evaluated the cost-effectiveness of IUI [7–10]. The study comparing expectant management to IUI showed that 3 years after the initial randomization of 6 months of expectant management to 6 months of IUI, there was no difference in pregnancy rate [7]. Immediate IUI was twice as expensive as expectant management.

The studies on IVF and IUI are difficult to compare, because they differ in patient characteristics, included cost, and treatment strategies [8–10]. Important omissions in these studies are the lack of including the cost of multiple gestations and clearly reporting the prognostic profile of the couples.

The prognostic profile of the couples is important as an indicator for the probability to conceive. In the FAST study, 14 % of the pregnancies were treatment independent [8]. In the study comparing a treatment algorithm to IVF, 32 % of all pregnancies occurred without active treatment [9]. The study comparing IUI with IVF reported a natural conception of 7 %, but in this study couples were included with a long duration of infertility, 46.5–53.4 months [10].

Data on expectant management in couples with a good prognosis for natural conception so far indicate that this is the first-line treatment. Whether expectant management should be followed by IUI or IVF is unclear. The cost-effectiveness studies published show that starting with IUI with or without ovarian stimulation is the first treatment choice, but for how long it should be continued is unclear [9, 10].

On the other hand, a randomized controlled trail comparing IVF eSET to three cycles of IUI with ovarian stimulation in couples with an unfavorable prognosis for natural conception showed that these two strategies could be equal, but no cost-effectiveness analysis was performed [11]. A follow-up of this study, the INeS-trial, is currently being performed in couples with a poor prognosis for natural conception with mild male or unexplained infertility [12]. This study compares six cycles of IUI, three cycles of IVF eSET, or six cycles of modified natural cycle IVF. In a modified natural cycle the oocyte that develops spontaneously is used for IVF. The cycle is minimally modified with a GnRH antagonist to prevent untimely ovulations, together with FSH to prevent collapse of the follicle and a concomitant fall in estradiol levels. Primary outcome is live birth of a child and also a cost-analysis will be performed. This study may give the final answer about which strategy is the most cost-effective strategy for couples after expectant management failed.

14.4 Conclusions

Current evidence on cost-effectiveness studies shows that treatment of a couple with a favorable prognosis should start with expectant management for at least 6 months. If the prognosis is poor, one can initiate treatment [7]. Despite the differences between all randomized controlled trials, especially the important omission of the prognostic profile, current evidence shows that IUI as an initial treatment for infertility is more cost-effective. The duration of treatment with IUI before proceeding to IVF and the additional value of ovarian stimulation to IUI are unclear. The INeS-trial could give the final answer on which treatment is the most cost-effective after expectant management and could prove whether or not IUI is a valuable and cost-effective treatment.

References

1. Van Voorhis BJ, Sparks AE, Allen BD, et al. Cost-effectiveness of infertility treatments: a cohort study. Fertil Steril. 1997;67(5):830–6.
2. NCCWCH. Clinical Guideline 11. Fertility: assessment and treatment for peole with fertility problems. 2004. http://www.nice.org.uk.
3. RCOG. The management of infertility in secondary care. 1998. http://www.rcog.org.uk.
4. Quaas A, Dokras A. Diagnosis and treatment of unexplained infertility. Rev Obstet Gynecol. 2008 Spring;1(2):69–76.
5. Hunault CC, Habbema JD, Eijkemans MJ, et al. Two new prediction rules for spontaneous pregnancy leading to live birth among subfertile couples, based on the synthesis of three previous models. Hum Reprod. 2004;19(9):2019–26.

6. Steures P, van der Steeg JW, Hompes PG, et al. Intrauterine insemination with controlled ovarian hyperstimulation versus expectant management for couples with unexplained subfertility and an intermediate prognosis: a randomised clinical trial. Lancet. 2006;368(9531):216–21.
7. Custers IM, van Rumste MM, van der Steeg JW, et al. Long-term outcome in couples with unexplained subfertility and an intermediate prognosis initially randomized between expectant management and immediate treatment. Hum Reprod. 2012;27(2): 444–50.
8. Reindollar RH, Regan MM, Neumann PJ, et al. A randomized clinical trial to evaluate optimal treatment for unexplained infertility: the fast track and standard treatment (FASTT) trial. Fertil Steril. 2010;94(3): 888–99.
9. Karande VC, Korn A, Morris R, et al. Prospective randomized trial comparing the outcome and cost of in vitro fertilization with that of a traditional treatment algorithm as first-line therapy for couples with infertility. Fertil Steril. 1999;71(3):468–75.
10. Goverde AJ, McDonnell J, Vermeiden JP, et al. Intrauterine insemination or in-vitro fertilisation in idiopathic subfertility and male subfertility: a randomised trial and cost-effectiveness analysis. Lancet. 2000;355(9197):13–8.
11. Custers IM, Konig TE, Broekmans FJ, et al. Couples with unexplained subfertility and unfavorable prognosis: a randomized pilot trial comparing the effectiveness of in vitro fertilization with elective single embryo transfer versus intrauterine insemination with controlled ovarian stimulation. Fertil Steril. 2011;96(5):1107–11.e1.
12. Bensdorp AJ, Slappendel E, Koks C, et al. The INeS study: prevention of multiple pregnancies: a randomised controlled trial comparing IUI COH versus IVF e SET versus MNC IVF in couples with unexplained or mild male subfertility. BMC Womens Health. 2009;9:35.

Vitrification of Human Oocytes and Embryos: An Overview

15

Juergen Liebermann

15.1 Introduction: The Importance of Cryopreservation for Reproductive Biology

Successful cryopreservation of gametes in animal reproduction has a track record of over 60 years. In human IVF, interest in cryobiology increased dramatically in 1983 following the first successful pregnancy after transfer of a human embryo [1]. In 1984, the first live birth following embryo cryopreservation was reported in Australia, which was followed 2 years later by another such birth in the USA. Currently, controlled ovarian hyperstimulation protocols commonly provide embryos in excess of those needed for fresh transfer. Therefore, techniques have been developed to store these surplus embryos in liquid nitrogen (referred to as cryopreservation) for an indefinite period of time without a significant compromise in their quality. Embryo cryopreservation and cryostorage is now a routine part of services offered at clinics treating infertility worldwide. With improvements in cryopreservation techniques and methods over the last three decades, the process has increasingly become an important part of the IVF process. By improving the cumulative pregnancy rate per oocyte retrieval [2], and by reducing the number of embryos transferred, thereby reducing the risk of multiple pregnancies [3], the technology brings two extremely important contributions to the field. According to data from the Centers for Disease Control and Prevention (CDC) from 2010, about 21.5 % of all IVF cycles in the USA used frozen embryos for transfer. In addition, data from the same registry compared live births per transfer in patients under 35 using frozen and fresh embryos (38.4 % versus 47.6 %, respectively) clearly showing that cryopreservation is an important adjunct to maximize the efficiency of every single patient's oocyte retrieval. However, it is important to note that clinical success with cryopreservation seems to be highly variable from lab to lab and may depend on many factors, which include the following:

1. Patient age
2. Ovarian stimulation protocol
3. Quality of embryos selected for freezing (*Scoring system*)
4. The developmental stage at freezing
5. The embryo culture system
6. Choice of cryoprotectants, concentration, and method of use
7. Parameters of the freezing/cooling and thawing/warming procedures
8. Cryopreservation protocol (traditional slow or vitrification)

The fundamental objectives for successful cryostorage of cells in liquid nitrogen at −196 °C can be summarized as follows (1) avoiding ice crystal formation during the cooling and freezing

J. Liebermann, Ph.D., H.C.L.D. (✉)
In Vitro Fertilization Laboratory, Fertility Centers of Illinois, River North Center, 900 N Kingsbury, Suite RW6, Chicago, IL 60610, USA
e-mail: Juergen.Liebermann@integramed.com

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_15,

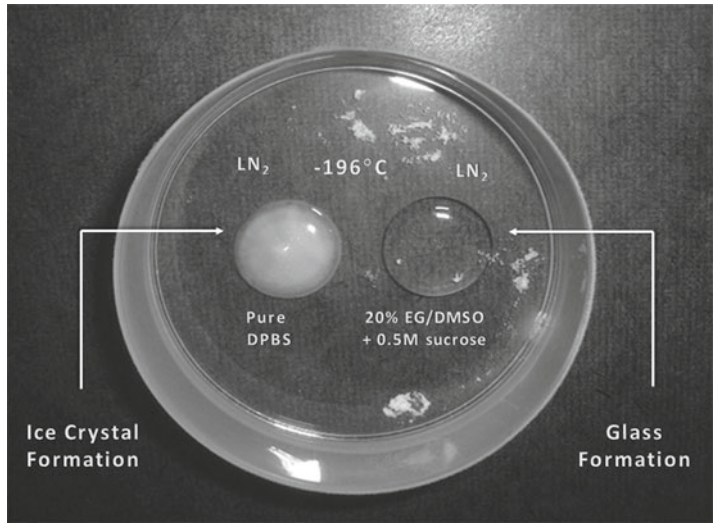

Fig. 15.1 Vitrification is the solidification of a solution ("*glass formation*") without ice crystallization. Two droplets of different solutions plunged directly into liquid nitrogen: the left droplet is pure Dulbecco's phosphate-buffered saline (DPBS) with ice crystallization; in contrast the right droplet contains an equimolar combination of 15 % ethylene glycol and dimethyl sulfoxide with 0.5 M sucrose in DPBS without ice crystallization (i.e., "glassification" in the vitrified state)

phases, (2) arresting cellular metabolism, but in a reversible way, (3) maintaining the structure and integrity of the DNA, (4) acceptable survival rates post warming and maintaining the developmental competence thereafter, and (5) the reliability of the technique. The most critical of these objectives is the avoidance of ice formation and all freezing protocols address this issue. In fact, a debated topic in the area of reproductive cryobiology is whether slow-cooling or rapid-cooling protocols both satisfy the fundamental cryobiological principles for reduction of damage by ice crystals during cooling and warming, and which approach is better. In any case, both methods include five principal steps (1) initial exposure to the cryoprotectant (CPA) to induce dehydration, (2) cooling to the temperature of liquid nitrogen (LN2; −196 °C), (3) storage in specially designed tanks containing LN2, (4) rehydration of the material (gametes or embryos) removing CPAs and replacement of the intracellular fluid at a precise rate, and (5) recovery and return to a physiological environment.

One way to achieve an ice crystal free cell is to establish a glassy or vitreous state with the use of ultra-high cooling rates and high CPA concentrations in the process of vitrification (Fig. 15.1). Vitrification is the achievement of a "state of suspended animation" wherein molecular translational motions are arrested without structural reorganization of the liquid. Vitrification protocols are relatively simple for the practitioner, potentially faster, and inexpensive. The process relies on the placement of the cell in a very small volume of vitrification medium that must be cooled at extreme rates, which are difficult to obtain in regular enclosed cryostraws or vials. The importance of using a very small volume, also referred to as the "minimal volume approach," was first described and published in 2005 [4, 5] using a carrier (device) called a cryotop, which facilitated the use of very small volumes of fluid for vitrification of embryos. Improvements in our understanding of the physical and biological principles of vitrification have led to numerous successful clinical applications, including more recently the vitrification of human oocytes. Vitrification protocols have in recent years become more established due to their many advantages, such as greater reliability and consistency in cryosurvival when compared to traditional slow freezing protocols. As of today, all developmental stages of the human embryo cultured in vitro have been successfully vitrified and

warmed, with resulting offspring. Vitrification techniques are therefore becoming a mainstream part of the IVF process [6, 7].

In achieving a vitrified state, the rate of cooling and the concentrations of CPA required for successful transition from living cell to glass-like solid are intimately linked. Achieving a higher cooling rate allows for the use of less CPA and vice versa. A primary strategy for vitrifying cells and tissue therefore is to increase the speed of thermal conductivity (cooling), while decreasing the concentration of the CPAs to reduce their potential toxicity to cells. However, it is important to know that every cell seems to require its own optimal cooling rate, e.g., mature unfertilized oocytes are much more sensitive to cryoinjury than any of the cell stages of the preimplantation embryo. The earliest attempts using vitrification as an ice-free cryopreservation method for embryos were reported in 1985 [8]. In 1993 successful vitrification of mouse embryos was demonstrated [9]. Furthermore, bovine oocytes and cleavage-stage embryos were vitrified and warmed successfully a few years later [10]. In 1999 and 2000 successful pregnancies and deliveries after vitrification and warming of human oocytes were reported [11, 12]. Since that time, human oocytes and blastocysts seem to have fared better after vitrification since both entities appear to be especially chill-sensitive and survive well when vitrified [13]. Vitrification media are CPA solutions that do not freeze when cooled at high cooling rates to a very low temperature (i.e., no ice crystals are formed). Consequently, interest in vitrification has risen dramatically as evidenced by the almost exponential growth in scientific publications about vitrification of gametes and embryos. And while the rate of cooling and warming and the concentration of the cryoprotectant required to achieve vitrification are inversely related, recent publications have shown the dominance of the warming rate over the cooling rate in the survival of oocytes subjected to a vitrification procedure [14, 15]. It appears that the warming rate must be faster than the cooling rate in order to avoid ice crystal formation during warming [16], but since the cooling rate and CPA concentration are also related, careful protocol design is important. Moderately high cooling rates allow for reasonably low CPA concentrations, while allowing for still higher warming rates.

It seems that a major concern with vitrification is this use of high CPA concentrations and therefore an unintentional negative impact (toxicity), which may affect the oocyte or embryo and its subsequent development in utero. The relatively high concentration of CPAs used for vitrification, and their known biological and physiochemical effects, may suggest that the toxicity of these agents is a key limiting factor in cryobiology. Not only does this toxicity prevent the use of fully protective (high) levels of these additives, but it may also manifest in the form of cryoinjury above and beyond that seen with classical causes of cell damage (osmotic shock due to water loss and ice formation) during cryopreservation. It is therefore essential to achieve a fine balance between the speed of cooling and the concentration of the vitrifying CPAs. This is also necessitated by the practical limit in the rate of cooling that can be achieved and the biological limit of tolerance of the cells to CPAs. One way around the problem of the high CPA concentration required for vitrification is to use two or more individual CPAs, each used at lower nontoxic levels, but together achieving the concentration required for successful glass formation with practical cooling rates. Recent publications [17–20] have confirmed the usefulness of this approach by using 15 % (vol/vol) ethylene glycol (EG) used in an equimolar mixture with dimethyl sulfoxide (DMSO) to vitrify human blastocysts. No negative effects on the perinatal outcomes from blastocyst transfers following vitrification and warming were seen when compared to those from fresh blastocyst transfers. In the warming procedure, the use of a nonpermeating agent, sucrose, acted as an osmotic buffer to reduce the osmotic shock to the blastocysts during rehydration. During warming, using a high extracellular concentration of sucrose (e.g., 1.0 M) counterbalanced the high concentration of CPAs and low water concentration in the cells, reducing the difference in osmolarity between the intra- and extracellular compartments. The high sucrose concentration cannot

totally prevent the cell from swelling due to influx of water, but it can reduce the speed and magnitude of swelling [6, 7, 21].

15.2 Vitrification of Human Oocytes

More recently, vitrification as an alternative method to traditional slow freezing has been shown to provide a high degree of success with metaphase-II human oocytes (Fig. 15.2). Historically, oocytes have been much more difficult to freeze than embryos, and routine oocyte cryopreservation has only emerged in recent years. The question arises as to what makes oocytes so unique and so difficult to freeze, when compared to embryos, which have been routinely frozen since the 1980s. As well as differences in cell size and membrane permeability, oocytes have a much lower volume-to-surface area ratio; hence, they are less efficient at taking up CPA and at losing water. Other differences to be considered are (a) the maternal DNA is held suspended in the cytoplasm on the meiotic spindle and not within the protective confines of a nuclear membrane, potentially exposing the DNA and microtubules to physical and chemical damage, (b) the oocyte is arrested in a delicate state primed for activation, and (c) changes in its environment can cause parthenogenetic activation.

An important application for oocyte freezing is the preservation of fertility in women with malignant/premalignant conditions who would have to undergo cytotoxic therapies that could deplete and/or kill their store of oocytes. Also important is its use by women who may want to delay childbearing ("clock-tickers") because of their careers or partnership status. Other applications include anonymous oocyte banking for donation, avoiding the creation of too many embryos that may never be used during IVF treatment, and saving oocytes for later use if a male partner is unable to collect a sperm sample on the day of his partner's oocyte retrieval. Oocyte cryostorage also offers an opportunity to reduce number of embryos generated in an IVF cycle, lessening the pressure on the patient to increase the number of embryos transferred.

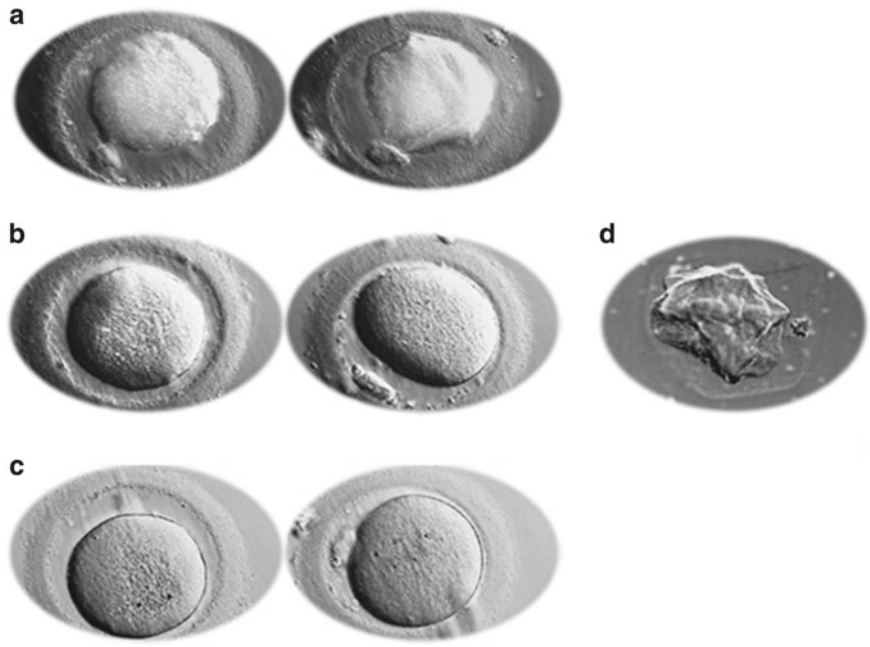

Fig. 15.2 Oocyte vitrification. (Row **a**) Oocytes exposed to 7.5 % EG/DMSO for 3 min; oocytes starting to shrink. (Row **b**) Oocytes exposed for additional 3 min in 7.5 % EG/DMSO; oocytes start to reexpand. (Row **c**) Oocytes for 7 more min in 7.5 % EG/DMSO; oocytes gained 80–90 % of their original volume back. (**d**) Oocyte exposed to 15 % EG/DMSO for 60 s

Babies born after oocyte cryopreservation have, to date, shown no apparent increase in congenital anomalies when compared to those born after the use of fresh oocytes. Thirteen years after the first slow-freeze birth, the number of babies born from vitrified oocytes is approaching that from slow-frozen oocytes without any noted increased risk of abnormality [22]. Reassuringly, Larman et al. [23] have shown that the meiotic spindle, an organelle in oocytes that is particularly sensitive to insult, in mouse and human oocytes is maintained after vitrification. Furthermore, cytogenetic analysis of embryos from fresh, and vitrified and warmed oocytes, shows no increase in anomalies [24]. Vitrification of oocytes does not appear to increase the risk of abnormal imprinting or disturbances in spindle formation or chromosome segregation [25]. In addition, no significant increase in abnormalities has been reported from pregnancies from cryostored oocytes [26, 27], regardless of the historical concerns that cryopreservation of mature oocytes might disrupt the meiotic spindle and thus increase the potential for aneuploidy in the embryos arising from such oocytes. Vitrification of oocytes, when applied to patients, will be a useful technology in reproductive medicine practice and will constitute a major step forward in Assisted Reproductive Technology (ART) [12, 28–30]. The scientific literature on oocyte cryopreservation grows daily and recently, Dominguez et al. [31] have shown that vitrification of oocytes does not disturb embryonic metabolomic profiles. Rienzi et al. [32] showed consistent and predictable delivery rates from vitrified oocytes in a longitudinal cohort multicentric study. Most publications however focus on clinical pregnancy rates [33, 34], and while this data is helpful to increase our confidence in the technology, it does little to direct new research on oocyte cryopreservation.

15.2.1 Human Pronuclear Embryos (Day 1 Postinsemination)

Using a traditional slow-freezing protocol, it takes approximately 100 min to cryopreserve a group of fertilized oocytes (zygotes) for a patient. Vitrification of the same zygotes can be accomplished in a much shorter time, by dividing them into small groups of 2–4, which each take about 12 min to vitrify. Staggering of groups, i.e., starting a second group while the first group is equilibrating in the CPA solution saves further time. Successful vitrification of zygotes with high survival after warming (~90 %), good cleavage rates on Day 2 (>80 %), and a blastocyst formation rate of 31 %, with ongoing pregnancies, have been reported [35–38]. Zygote vitrification implemented in a clinical setting can provide a clinical pregnancy rate of close to 30 %, with an implantation rate of 17 % [38]. The pronuclear stage appears tolerant of the vitrification and warming process, perhaps due to the significant membrane permeability changes that occur postfertilization. Such changes to the oolema may also make the cell more stable and able to cope with the vagaries of cold-shock and the striking osmotic fluctuations that occur during the vitrification process.

15.2.2 Cleavage Stage Embryos (Day 2–4 Postinsemination)

Liebermann and Tucker [21], using either the cryoloop (Vitrolife, Denver, CO) or the hemi-straw system (HSV; Irvine Scientific, Santa Ana, CA), showed postwarming survival rates (defined as >50 % of blastomeres intact in Day 3 embryos 2 h post warming) from 84 to 90 % depending on the carrier system used. There was a reasonable further cleavage and a compaction rate of 34 %. This finding supports previous reports in which high survival rates from 8-cell human embryos using 40 % EG were documented [39]. More recently, reported successful pregnancies and deliveries after vitrification of Day-3 human embryos using an open pulled straw (OPS) as a carrier device have been reported [40, 41]. Their results showed a negative correlation between stage of development and survival. Eight-cell embryos showed a higher survival rate (79.2 %; 62/78) than did embryos with fewer than six cells (21.1 %; 11/53) after vitrification. Despite the fact that Liebermann and Tucker [21] achieved a promising postwarming survival rate, overall only about 34 % of the surviving embryos had the developmental potential to reach the

compaction stage. Loutradi et al. [42] performed a meta-analysis and systematic review by comparing slow freeze and vitrification protocols for cleavage stage embryos and found a survival rate of 84.0 % and 97.0 %, respectively. In addition, clinical pregnancy rates between 35 and 48 %, with implantation rates between 15 and 39 %, have been reported following vitrification of cleavage stage embryos [43–46]. So clearly vitrification appears to have a positive impact on overall embryo utilization. A study on the neonatal outcomes of 907 vitrified/warmed cleavage stage embryos found no significant increase in the congenital birth defect rate when compared with pregnancies using fresh cleavage stage embryos [47]. And recently, Cobo et al. [48] observed no negative effect of vitrification of early cleavage stage embryos on the survival and delivery rates. Shi et al. [49] further supported this approach showing no significant differences in obstetrical and neonatal outcomes when comparing fresh and vitrified Day 3 embryo transfer procedures.

15.2.3 Blastocyst Stage Embryos (Day 5, 6, and 7 Postinsemination)

It has been established that the activation of the embryonic genome occurs after the 8-cell stage (*3-day postoocyte retrieva*l) is reached in humans [50]. However, if activation does not occur, it is unlikely that the embryo will survive for very long. Therefore, improvement in human IVF outcomes might be achieved by identifying those embryos that will progress beyond the 8-cell stage. Blastocyst culture (*to 5-day postoocyte retrieval*) allows the transfer of embryos that likely have an activated embryonic genome. Furthermore, it has been shown that Day 3 morphology can only predict approximately 48 % of those embryos that will eventually form blastocysts suitable for use on Day 5/6 [51]. In order to best identify the best embryos in a group therefore, Day 3 morphology is unlikely to be useful, especially for patients with many embryos. Extended culture beyond the 8-cell stage may be a better selection mechanism, since the less viable embryos will tend to arrest in development early on, "selecting" themselves as noncandidates for fresh transfer or *cryopreservation*. This process may eliminate a lot of embryos from being used, but the net result is that the chance of achieving a pregnancy is potentially improved. In addition, at the blastocyst stage, a lower number of embryos can be transferred since there is more confidence in embryo viability, resulting in less high-order multiple pregnancies. This is also true for cryopreserved blastocysts, which give higher pregnancy and implantation rates per thawed embryo transferred. Furthermore, the higher cell number at this stage allows better compensation for cryoinjuries and may result in greater viability and faster recovery.

The success of blastocyst stage transfers in increasing pregnancy rates by better identifying the best embryos in a cohort has led to a trend toward culturing embryos to this stage instead of performing transfer on Day 2 or 3 post oocyte retrieval. Blastocyst culture also allows for the transfer of embryos that likely have an activated embryonic genome. Blastocysts are more likely to implant compared with Day 3 cleavage stage embryos and give the confidence to transfer a single embryo to minimize the risks associated with multiple pregnancies, while still maintaining an overall high chance of pregnancy. With the ever-increasing production of blastocysts on the 5th, 6th, and 7th day of embryo culture, and the trend toward transferring fewer, a successful cryopreservation program for blastocysts is becoming an increasingly important part of ART. And when asking patients to transfer just a single embryo, having the insurance of a successful cryopreservation program can be the key to having the confidence to transfer embryos one at a time. Vitrification can provide such confidence since survival rates for human blastocysts are between 70 and 90 % using different carriers, with clinical pregnancy rates of 37–53 % and implantation rates of 20–30 % [18–20, 52–59].

15.2.4 The Blastocyst Vitrification Procedure in General

Prior to vitrification, certain steps such as dehydration of the embryo have to be performed.

Blastocysts are immersed in a hypertonic CPA solution, which will cause an immediate efflux of water by osmosis. Given that cell membranes are more permeable to water than to CPAs (since the water molecule is smaller), an immediate cellular contraction and shrinkage occurs, followed by the slow entry of the CPA [60]. As equilibrium is achieved, the cellular volume is nearly restored as the CPA enters the cells. However, a feature unique to the blastocyst, a fluid-filled cavity called the blastocoele, can dilute CPAs and limit their effectiveness. A decrease in survival rate after vitrification has been noted when the volume of the blastocoelic cavity is increased. The reason for this is likely due to an insufficient permeation of CPA through the blastocoele and into surrounding cells. Also, residual water in the blastocoele cavity may increase the potential for ice crystal formation during cooling, thus reducing the postwarming survival. Vanderzwalmen et al. [59] showed that survival rates in cryopreserved, expanded blastocysts could be improved by artificial reduction of the blastocoele cavity, and others also consider that blastocoele collapse is necessary previtrification on whatever the day the blastocyst forms [57, 61].

One study has suggested that no significant differences in viability, implantation potential, or pregnancy outcome are observed when blastocysts are frozen on Day 5 versus Day 6 [62]. However, our "body of data" refutes the comparable implantation rate for blastocysts cryopreserved on Day 5 or 6. Our data suggest that the older blastocysts (Day 6) perform less well than Day 5 blastocysts, perhaps because of the likelihood of a larger blastocoele in these embryos. The literature on artificially collapsing (AC) the blastocoele is significant and perhaps suggested an opportunity that could potentially help us to improve the outcomes for Day 6 blastocysts. Reduction of the blastocoele using AC can be performed using different approaches such as microneedles, sucrose solutions, or laser [56, 61, 63–65]. In 2003 and 2004, two groups independently reported a beneficial effect of applying artificial collapsing to blastocysts prior to vitrification. Son et al. [63] observed a clinical pregnancy rate of 48 % and an implantation rate of 29 %. Hiraoka et al. [56] collapsed Day 5 and Day 6 blastocysts by manual pipetting the blastocyst and achieved clinical pregnancy of 50 % with an implantation of 33 %. Moreover, Mukeida et al. [61] found that the survival rate of vitrified blastocysts was negatively correlated with the expansion of the blastocoele. They speculated that a large blastocoele may disturb the efficacy of vitrification. They collapsed the blastocoele with a microneedle or by making a hole between two trophectoderm cells with a laser pulse. After collapsing the cavity in the blastocyst, the survival improved from 86 % to 97.2 %. Moreover, their pregnancy rate went up from 34.1 % to 60.2 % with an implantation rate of 46.7 %. More recently, another two publications have looked into the possible benefit of applying AC prior to vitrification. Iwayama et al. [64] used a laser pulse, or osmotic shock by exposing the blastocysts to sucrose, and the implantation rate was significant higher in both groups compared to the control group vitrified without AC (59.7 % and 49.3 % vs. 34.2 %). Hur et al. [65] also looked also at the effect of artificial shrinkage using a 29-gauge needle or a laser pulse on clinical outcomes in fresh transfers, and they observed a significant increase in the clinical pregnancy in the study group (+ AC; 58.8 %) compared to the control group (−AC; 39.0 %). All publications mentioned conclude that artificial collapsing has a beneficial effect on the clinical pregnancy as well as on the implantation rate both in frozen and fresh cycles.

15.2.5 Vitrification Procedure for Blastocysts at FCI

Fertility Centers of Illinois "IVF Laboratory River North" (Chicago) have performed blastocyst vitrification utilizing a "closed system" [HSV (High Security Vitrification Kit); CryoBio System, L'Aigle, France] and a two-step CPA incubation at 24 °C. Prior to vitrification, the blastocysts in the study group were put on an inverted microscope equipped with a laser system (ZILOS-tk, Hamilton Thorne), and one shot (100 % power, 500 μs pulse length) was applied at the junction between two trophectoderm cells in each blastocyst (Fig. 15.3). The blastocysts were then returned to the 37 °C incubator for

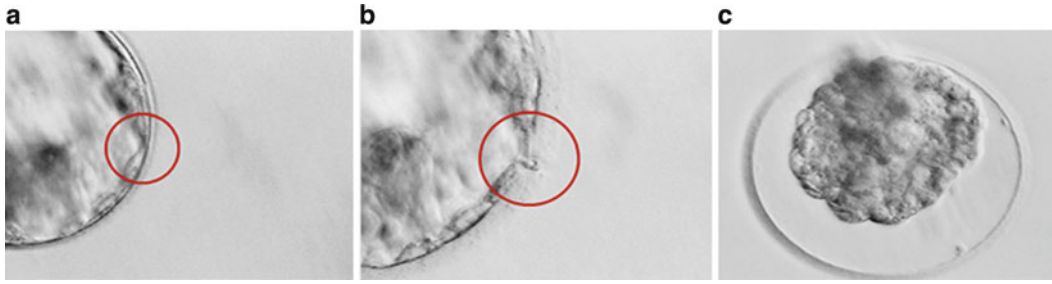

Fig. 15.3 Artificial collapsing of blastocysts in a 3-step procedure. (**a**) Locate junction between two trophectoderm cells, adjust laser to a power of 500 µs. (**b**) Shoot once at a junction between two trophectoderm cells and then put the blastocyst back into the incubator for 5 min. (**c**) Cavity is now collapsed and blastocyst ready to get vitrified

5–10 min to allow them to collapse. Blastocysts were then placed in equilibration solution (ES), which is the base medium (HEPES-buffered HTF with 20 % Synthetic Serum Substitute (SSS) containing 7.5 % (v/v) EG and 7.5 % (v/v) DMSO for vitrification. After 5–7 min, the blastocysts were washed quickly in vitrification solution (VS), base medium but containing 15 % (v/v) DMSO, 15 % (v/v) EG, and 0.5 M sucrose for 45–60 s and transferred onto the HSV using a micropipette. Immediately after the loading of not more than two blastocysts in a 1 µl drop of VS on the HSV, the straws were heat sealed, then plunged into LN2, and secondarily stored inside 5 mL liquid nitrogen prefilled canes (Visotube Rond, IMV, France).

To use the embryos and remove the CPAs, blastocysts were warmed and diluted in a three-step process. With the HSV submerged in LN2, the inner straw was removed, and then the carrier with the blastocysts was quickly placed directly into a prewarmed (37 °C) organ culture dish containing 1 mL of base medium with 1.0 M sucrose. Blastocysts were picked up directly from the HSV and placed in a fresh drop of the same solution at 24 °C and immediately connected with a drop containing just 0.5 M sucrose. After 5 min blastocysts were transferred to a fresh drop of the 0.5 M sucrose solution and connected with drops of base medium (without sucrose) for an additional 5 min. Finally, blastocysts were washed in the base medium for 3 min and returned to the culture medium (SAGE Blastocyst Medium, Trumbull, CT,) containing 20 % SSS until transfer.

Both natural and hormone replacement cycles seem to provide comparable levels of receptivity in naturally cycling women, though they differ in level of convenience. To calculate the day of transfer, we calculated the "*day of ovulation*" (whether in a "natural" or "artificial" transfer cycle), then thawed and transferred 1–2 blastocysts on the fifth day after ovulation. Regardless of the day of cryopreservation of the embryo (Day 5, 6, or 7) at thawing, all blastocysts were treated as if they had been frozen on the fifth day of development.

15.2.6 Blastocyst Vitrification with Artificial Collapsing Prior Vitrification Steps at FCI

Between January 2004 and October 2012, the *Fertility Centers of Illinois* "IVF Laboratory River North" (Chicago) have vitrified 17,529 blastocysts from 4,594 patients (Table 15.1). After almost 10 years of vitrifying blastocysts using an open as well as a closed carrier device, and 4,000 frozen embryo transfer (FET) cycles with an average number of 1.8 embryos transferred, the perinatal outcome is as follows: 1,056 babies born [532 girls and 524 boys (Table 15.2)] with no abnormalities recorded.

Between 2007 and September 2012 the *Fertility Centers of Illinois* "IVF Laboratory River North" (Chicago) performed 1,482 FETs without collapsing the blastocysts prior to vitrification (Group A). The mean age of the patients was 35.4

Table 15.1 Retrospective data from 4,594 patients (average age 34.0 ± 4.9) with blastocyst cryopreservation by vitrification from January 2004 till October 2012

Day of development	Day 5	Day 6	Day 7	Total
No. of blastocysts vitrified (%)	8,484 (48.5 %)	8,638 (49.3 %)	407 (2.3 %)	17,529

Table 15.2 Perinatal outcome of vitrified blastocysts after close to 4,000 transfers between 2004 and 2012

Day of development		Day 5	Day 6
Deliveries (Total)	855	496	359
Total babies born	1,056	628	428
Female	532	328	204
Male	524	300	224
Singletons	661 (77.0 %)	367 (74.0 %)	294 (82.0 %)
Twins	187 (22.0 %)	126 (25.5 %)	61 (17.0 %)
Triplets	7 (1.0 %)	3 (0.5 %)	4 (1.0 %)

Table 15.3 A comparison of retrospective data from the cryopreservation program (*Fertility Centers of Illinois*, *Chicago*) of vitrified blastocysts without AC (Group A) and with AC (Group B) using a closed carrier system from January 2007 till October 2012

Technique	Group A (– AC)	Group B (+ AC)
Patient's age (year)	35.3 ± 5.0	35.4 ± 4.9
No. of transfers	1,482	276
No. of blastocysts warmed	2,697	510
No. of blastocysts survived (%)	2,618 (98.6)	505 (99.0)
No. of blastocysts transferred	2,618	505
Mean no. of blastocysts transferred	1.8	1.8
No. of implantations (%)	836 (32.0)*	236 (46.7)*
No. of positive pregnancy/VET (%)	752 (50.7)**	207 (75.0)**
No. of clinical pregnancy/VET (%)	421 (43.2)**	175 (63.4)**
Ongoing/delivered pregnancies (%)	518 (35.0)**	162 (58.7)**

VET vitrified embryo transfer
Values are numbers unless otherwise described
*$P < 0.01$
**$P < 0.001$

± 4.9 years in this group, and an average of 1.8 embryos were transferred per FET. 276 FETs were performed using embryos where AC was performed prior to vitrification (Group B) with a mean age of 35.3 ± 5.0 years and also with an average of 1.8 embryos transferred (Table 15.3).

Recovery and survival of blastocysts was not significantly different between groups (99.8 % vs. 100 % recovery; 98.6 % vs. 98.9 % survival). However, there was a significant improvement in Group B compared with Group A for the following (a) *clinical pregnancy rate* (*cPR*): 63.4 % vs. 43.1 %, (b) *ongoing pregnancy* (*oPR*): 58.7 % vs. 35.0 %, and (c) *implantation rate* (*IR*): 46.7 % vs. 32.0 % (17.3.).

When the vitrified-warmed blastocysts were divided into Day 5 and Day 6 groups, the following outcomes were observed (Table 15.4): in 863 FETs transferring Day 5 blastocysts from Group A (mean age of 35.1 ± 5.1), the IR, cPR, and oPR were 36.5 %, 48.1 %, and 39.5 % compared to 52.4 %, 68.1 %, and 64.4 % of Day 5 blastocysts

Table 15.4 A comparison of retrospective data from the cryopreservation program (*Fertility Centers of Illinois, Chicago*) of vitrified Day 5 and Day 6 blastocysts without AC (Group A) and with AC (Group B) using a closed carrier system from January 2007 till October 2012

	Group A (– AC)		Group B (+ AC)	
Technique	Day 5	Day 6	Day 5	Day 6
Patient's age (year)	35.1 ± 5.1	35.5 ± 4.9	35.1 ± 4.6	35.7 ± 5.0
No. of transfers	863	619	135	141
No. of blastocysts warmed	1,553	1,144	250	260
No. of blastocysts survived (%)	1,538 (99.0)	1,122 (98.1)	248 (99.2)	258 (99.2)
No. of blastocysts transferred	1,511	1,107	246	256
Mean no. of blastocysts transferred	1.8	1.8	1.8	1.9
No. of implantations (%)	551 (36.5)*	285 (25.7)***	129 (52.4)*	107 (41.8)***
No. of positive pregnancy/VET (%)	485 (56.2)**	267 (43.1)****	106 (78.5)**	101 (71.6)**
No. of clinical pregnancy/VET (%)	415 (48.1)**	225 (36.3)****	92 (68.1)**	83 (58.9)****
Ongoing/delivered pregnancies (%)	341 (39.5)**	177 (28.9)****	87 (64.4)**	75 (53.2)****

VET vitrified embryo transfer
Values are numbers unless otherwise described
Day 5: *$P<0.01$; **$P<0.001$; Day 6: ***$P<0.01$; ****$P<0.001$

Table 15.5 A comparison of retrospective data from the cryopreservation program (*Fertility Centers of Illinois, Chicago*) of vitrified Day 5 and Day 6 blastocysts without AC (Group A) and with AC (Group B) using a closed carrier system in patients younger than 35 years old from January 2007 till October 2012

	Group A (– AC) Less than 35 years old		Group B (+ AC) Less than 35 years old	
Technique	Day 5	Day 6	Day 5	Day 6
Patient's age (year)	31.2 ± 2.4	31.5 ± 0.6	31.5 ± 2.1	30.6 ± 2.8
No. of transfers	438	287	68	54
No. of blastocysts warmed	796	543	123	102
No. of blastocysts survived (%)	782 (98.2)	530 (97.6)	122 (99.2)	101 (99.0)
No. of blastocysts transferred	768	521	122	101
Mean no. of blastocysts transferred	1.8	1.8	1.8	1.9
No. of implantations (%)	312 (40.6)*	155 (29.8)**	77 (63.1)*	45 (44.6)**
No. of positive pregnancy/VET (%)	254 (58.0)*	140 (48.8)**	57 (83.8)*	42 (77.8)**
No. of clinical pregnancy/VET (%)	225 (51.4)*	119 (41.5)**	52 (76.5)*	36 (66.7)**
Ongoing/delivered pregnancies (%)	341 (44.7)*	100 (34.8)**	50 (73.5)*	32 (59.3)**

VET vitrified embryo transfer
Values are numbers unless otherwise described
Day 5: *$P<0.001$; Day 6: **$P<0.001$

from Group B (mean age of 35.1 ± 4.6). As shown in Table 15.4, implantation, cPR, and oPR occurring in the Day 5 blastocysts from Group B were significantly higher than in the Day 5 blastocyst from Group A (χ^2; $P<0.001$, respectively).

Comparing Day 6 frozen blastocysts in Group A (mean age of 35.5 ± 4.9) with Day 6 outcomes in Group B (mean age of 35.7 ± 5.0), the following data for implantation, cPR, and oPR was observed: 25.7 %, 36.3 %, and 28.9 % vs. 41.8 %, 58.9 %, and 53.2 %, respectively (Table 15.4). As shown in Table 15.4, implantation, cPR, and oPR occurring in the Day 6 blastocysts of Group B were significantly higher than those for Day 6 blastocysts from Group A (χ^2; $p<0.001$ for each comparison).

Table 15.5 shows a summary of the results for patients under 35 without AC (Group A; $n=717$) and with AC (Group B; $n=124$). The following cPRs, oPRs, and IRs were observed between Groups A and B, respectively: 48.0 % vs. 71.8 %, 41.0 % vs. 68.5 %, and 36.4 % vs. 53.1 %. The same trend and tendency was seen for all other age groups (Table 15.5).

15.3 Summary

An improving awareness of vitrification technology and the continuous improvement and refinements of the techniques has allowed vitrification to become a preferred method for the preservation of human oocytes and embryos. The growing number of reports of successfully completed pregnancies following vitrification makes for an extremely encouraging future for its wider adoption, the procedure appears safe, is easily assimilated into a busy IVF laboratory, and promises improved patient outcomes when used correctly.

For blastocyst vitrification, a closed system is effective for achieving high implantation and pregnancy rates as seen with fresh embryo transfers.

Although the implantation and clinical pregnancy outcomes have historically been significantly different when comparing Day 5 and Day 6 blastocysts, our data should now encourage cryopreservation of Day 6 blastocysts which can be expected to do well. Based on the data presented, it is clear that the vitrification of Day 6 blastocysts is of clinical value since it can result in live births at high rates. This observation is in contrast to that of Saphiro et al. [66] and Levens et al. [67] who showed that the blastocyst development rate impacts outcomes in slow cryopreserved blastocyst transfer cycles. Our data now show that artificial shrinkage of the blastocoele, releasing fluid from the cavity prior vitrification, significantly improves pregnancy rates and implantation. This increase was observed in Day 5 as well as Day 6 blastocysts. In addition, the increase in outcome was observed in all patients regardless of age. Artificial collapsing of Day 5 and Day 6 blastocysts prior to the steps of vitrification is beneficial for all outcome parameters including clinical and ongoing pregnancy rate as well as implantation rate.

In summary, comparing no AC with AC prior vitrification, an average increase for all ages in regard to clinical (40 % vs. 58 %), ongoing pregnancy rates (30 % vs. 53 %), and implantation (28 % vs. 41.5 %) were observed. In regard to day of embryo development, comparing no AC with AC, an average increase for Day 5 and Day 6 were established for the following:

- Clinical (Day 5: 48.1 % vs. 68.1 %; Day 6: 36.1 % vs. 58.9 %)
- Ongoing pregnancy rates (Day 5: 39.5 % vs. 64.4 %; Day 6: 28.9 % vs. 53.2 %)
- Implantation (Day 5: 36.5 % vs. 52.4 %; Day 6: 25.7 vs. 41.8 %)

Our data has shown that freezing at the blastocyst stage provides excellent survival, implantation, and clinical pregnancy rates [18–20]. The following considerations apply to a blastocyst vitrification program (a) without a successful blastocyst vitrification program, extended culture of embryos is not recommended, (b) the blastocyst is composed of many cells and therefore may be better able to compensate for cryoinjury, (c) the cells are smaller thus making cryoprotectant penetration faster, and (d) on average fewer blastocysts per patient are cryostored, but each one when thawed has a greater potential for implantation, often with an opportunity for an FET with a single blastocyst.

15.4 Conclusions

In conclusion, vitrification of oocytes and all stages of the pre-implantation human embryo is a viable and feasible alternative to traditional slow-freezing methods with an ever-increasing clinical track record [68]. Note however that a standardized vitrification protocol applicable to oocytes and preimplantation embryo may not be realistic because of:

(a) Different volume-to-surface area ratio for oocytes and all preimplantation stages
(b) Differing cooling rate requirements for oocytes, zygotes, cleavage stage embryos, and blastocysts
(c) Variable chill-sensitivity between these different developmental stages

Currently, a widely used protocol that can be applied to any embryo stage is the two-step equilibration in an equimolar combination of the cryoprotectants EG and DMSO, at a concentration of 15 % each (v/v) supplemented with 0.5 mol/L sucrose. This solution is safe for clinical use, giving rise to healthy babies without abnormalities

regardless of the developmental stage being vitrified.

For the adoption of vitrification in ART, as with all new technologies, there has been resistance, but as clinical data have been accrued, this technology is becoming more commonly adopted as standard procedure in many IVF programs worldwide. With this increased use of vitrification in human-assisted reproduction, we should see an evolution of the vitrification process as it is fine-tuned to clinical needs. Thus, the future should bring higher levels of clinical efficiency, utilization, and universal acceptance.

Acknowledgment The author acknowledges a friend and colleague Dr. Joe Conaghan (Pacific Fertility Center; San Francisco) for reviewing and editing the manuscript and thanks the physicians and the embryologists (Elissa Pelts, BS; Jill Matthews, BS; Sara Sanchez, BS; Yuri Wagner, BS; Rebecca Brohammer, BS; and Ewelina Pawlowska, MS) at the Fertility Centers of Illinois (Chicago) for their invaluable contributions and support in pushing vitrification to become the standard protocol for cryopreservation of human oocytes and blastocysts within the program since 2004.

References

1. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature. 1983;305(5936):707–9.
2. Bergh C, Josefsson B, Nilsson L, et al. The success rate in a Swedish in-vitro fertilization unit: a cohort study. Acta Obstet Gynecol Scand. 1995;74(6):446–50.
3. Tiitinen A, Halttunen M, Harkki P, et al. Elective single embryo transfer: the value of cryopreservation. Hum Reprod. 2001;16(6):1140–4.
4. Kuwayama M, Vajta G, Ieda S, et al. Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. Reprod Biomed Online. 2005;11(5):608–14.
5. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology. 2007;67:73–80.
6. Liebermann J, Nawroth F, Isachenko V, et al. Potential importance of vitrification in reproductive medicine. Biol Reprod. 2002;67(6):1671–80.
7. Liebermann J, Dietl J, Vanderzwalmen P, et al. Recent developments in human oocyte, embryo and blastocyst vitrification: where are we now? Reprod Biomed Online. 2003;7(6):623–33.
8. Rall WF, Fahy GM. Ice-free cryopreservation of mouse embryos at −196 degrees C by vitrification. Nature. 1985;313(6003):573–5.
9. Ali J, Shelton JN. Vitrification of preimplantation stages of mouse embryos. J Reprod Fertil. 1993; 98(2):459–65.
10. Vajta G, Holm P, Kuwayama M, et al. Open Pulled Straw (OPS) vitrification: a new way to reduce cryoinjuries of bovine ova and embryos. Mol Reprod Dev. 1998;51(1):53–8.
11. Kuleshova L, Gianaroli L, Magli C, et al. Birth following vitrification of a small number of human oocytes: case report. Hum Reprod. 1999;14(12): 3077–9.
12. Yoon TK, Chung HM, Lim JM, et al. Pregnancy and delivery of healthy infants developed from vitrified oocytes in a stimulated in vitro fertilization-embryo transfer program. Fertil Steril. 2000;74(1):180–1.
13. Walker DL, Tummon IS, Hammitt DG, et al. Vitrification versus programmable rate freezing of late stage murine embryos: a randomized comparison prior to application in clinical IVF. Reprod Biomed Online. 2004;8(5):558–68.
14. Seki S, Mazur P. The dominance of warming rate over cooling rate in the survival of mouse oocytes subjected to a vitrification procedure. Cryobiology. 2009;59(1):75–82.
15. Mazur P, Seki S. Survival of mouse oocytes after being cooled in a vitrification solution to −196 degrees C at 95 degrees to 70,000 degrees C/min and warmed at 610 degrees to 118,000 degrees C/min: a new paradigm for cryopreservation by vitrification. Cryobiology. 2011;62(1):1–7.
16. Fahy GM, Rall WF. Vitrification: an overview. In: Tucker MJ, Liebermann J, editors. Vitrification in assisted reproduction: a user's manual and troubleshooting guide. London: Informa Healthcare; 2007.
17. Takahashi K, Mukaida T, Goto T, et al. Perinatal outcome of blastocyst transfer with vitrification using cryoloop: a 4-year follow-up study. Fertil Steril. 2005;84(1):88–92.
18. Liebermann J, Tucker MJ. Comparison of vitrification and conventional cryopreservation of day 5 and day 6 blastocysts during clinical application. Fertil Steril. 2006;86(1):20–6.
19. Liebermann J. More than six years of blastocyst vitrification – what is the verdict? US Obstet Gynecol. 2011;5:14–7.
20. Liebermann J. Vitrification of human blastocysts: an update. Reprod Biomed Online. 2009;19 Suppl 4:4328.
21. Liebermann J, Tucker MJ. Effect of carrier system on the yield of human oocytes and embryos as assessed by survival and developmental potential after vitrification. Reproduction. 2002;124(4):483–9.
22. Noyes N, Porcu E, Borini A. Over 900 oocyte cryopreservation babies born with no apparent increase in congenital anomalies. Reprod Biomed Online. 2009;18(6):769–76.
23. Larman MG, Minasi MG, Rienzi L, et al. Maintenance of the meiotic spindle during vitrification in human and mouse oocytes. Reprod Biomed Online. 2007;15(6):692–700.

24. Cobo A, Rubio C, Gerli S, et al. Use of fluorescence in situ hybridization to assess the chromosomal status of embryos obtained from cryopreserved oocytes. Fertil Steril. 2001;75(2):354–60.
25. Trapphoff T, El Hajj N, Zechner U, et al. DNA integrity, growth pattern, spindle formation, chromosomal constitution and imprinting patterns of mouse oocytes from vitrified pre-antral follicles. Hum Reprod. 2010;25(12):3025–42.
26. Chian RC, Gilbert L, Huang JY, et al. Live birth after vitrification of in vitro matured human oocytes. Fertil Steril. 2009;91(2):372–6.
27. Cobo A. Oocyte vitrification: a watershed in ART. Fertil Steril. 2012;98(3):600–1.
28. Lucena E, Bernal DP, Lucena C, et al. Successful ongoing pregnancies after vitrification of oocytes. Fertil Steril. 2006;85(1):108–11.
29. Cobo A, Diaz C. Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril. 2011;96(2): 277–85.
30. Cobo A, Remohi J, Chang CC, et al. Oocyte cryopreservation for donor egg banking. Reprod Biomed Online. 2011;23(3):341–6.
31. Dominguez F, Castello D, Remohi J, et al. Effect of vitrification on human oocytes: a metabolic profiling study. Fertil Steril. 2013;99(2):565–572.e3.
32. Rienzi L, Cobo A, Paffoni A, et al. Consistent and predictable delivery rates after oocyte vitrification: an observational longitudinal cohort multicentric study. Hum Reprod. 2012;27(6):1606–12.
33. Boldt J, Tidswell N, Sayers A, et al. Human oocyte cryopreservation: 5-year experience with a sodium-depleted slow freezing method. Reprod Biomed Online. 2006;13(1):96–100.
34. Boldt J, Cline D, McLaughlin D. Human oocyte cryopreservation as an adjunct to IVF-embryo transfer cycles. Hum Reprod. 2003;18(6):1250–5.
35. Park SP, Kim EY, Oh JH, et al. Ultra-rapid freezing of human multipronuclear zygotes using electron microscope grids. Hum Reprod. 2000;15(8):1787–90.
36. Jelinkova L, Selman HA, Arav A, et al. Twin pregnancy after vitrification of 2-pronuclei human embryos. Fertil Steril. 2002;77(2):412–4.
37. Liebermann J, Tucker MJ, Graham JR, et al. Blastocyst development after vitrification of multipronuclear zygotes using the Flexipet denuding pipette. Reprod Biomed Online. 2002;4(2):146–50.
38. Al-Hasani S, Ozmen B, Koutlaki N, et al. Three years of routine vitrification of human zygotes: is it still fair to advocate slow-rate freezing? Reprod Biomed Online. 2007;14(3):288–93.
39. Mukaida T, Wada S, Takahashi K, et al. Vitrification of human embryos based on the assessment of suitable conditions for 8-cell mouse embryos. Hum Reprod. 1998;13(1O):2874–9.
40. El-Danasouri I, Selman H. Successful pregnancies and deliveries after a simple vitrification protocol for day 3 human embryos. Fertil Steril. 2001;76(2): 400–2.
41. Selman HA, El-Danasouri I. Pregnancies derived from vitrified human zygotes. Fertil Steril. 2002; 77(2):422–3.
42. Loutradi KE, Kolibianakis EM, Venetis CA, et al. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. Fertil Steril. 2008;90(1):186–93.
43. Rama Raju GA, Haranath GB, Krishna KM, et al. Vitrification of human 8-cell embryos, a modified protocol for better pregnancy rates. Reprod Biomed Online. 2005;11(4):434–7.
44. Desai N, Blackmon H, Szeptycki J, et al. Cryoloop vitrification of human day 3 cleavage-stage embryos: post-vitrification development, pregnancy outcomes and live births. Reprod Biomed Online. 2007;14(2):208–13.
45. Li Y, Chen ZJ, Yang HJ, et al. [Comparison of vitrification and slow-freezing of human day 3 cleavage stage embryos: post-vitrification development and pregnancy outcomes]. Zhonghua Fu Chan Ke Za Zhi. 2007;42(11):753–5.
46. Balaban B, Urman B, Ata B, et al. A randomized controlled study of human Day 3 embryo cryopreservation by slow freezing or vitrification: vitrification is associated with higher survival, metabolism and blastocyst formation. Hum Reprod. 2008;23(9):1976–82.
47. Rama Raju GA, Jaya Prakash G, Murali Krishna K, et al. Neonatal outcome after vitrified day 3 embryo transfers: a preliminary study. Fertil Steril. 2009; 92(1):143–8.
48. Cobo A, de los Santos MJ, Castello D, et al. Outcomes of vitrified early cleavage-stage and blastocyst-stage embryos in a cryopreservation program: evaluation of 3,150 warming cycles. Fertil Steril. 2012;98(5):1138–46.e1.
49. Shi W, Xue X, Zhang S, et al. Perinatal and neonatal outcomes of 494 babies delivered from 972 vitrified embryo transfers. Fertil Steril. 2012;97(6):1338–42.
50. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature. 1988;332(6163): 459–61.
51. Graham J, Han T, Porter R, et al. Day 3 morphology is a poor predictor of blastocyst quality in extended culture. Fertil Steril. 2000;74(3):495–7.
52. Yokota Y, Sato S, Yokota M, et al. Successful pregnancy following blastocyst vitrification: case report. Hum Reprod. 2000;15(8):1802–3.
53. Reed ML, Lane M, Gardner DK, et al. Vitrification of human blastocysts using the cryoloop method: successful clinical application and birth of offspring. J Assist Reprod Genet. 2002;19(6):304–6.
54. Mukaida T, Nakamura S, Tomiyama T, et al. Successful birth after transfer of vitrified human blastocysts with use of a cryoloop containerless technique. Fertil Steril. 2001;76(3):618–20.
55. Mukaida T, Takahashi K, Kasai M. Blastocyst cryopreservation: ultrarapid vitrification using cryoloop technique. Reprod Biomed Online. 2003;6(2): 221–5.

56. Hiraoka K, Kinutani M, Kinutani K. Blastocoele collapse by micropipetting prior to vitrification gives excellent survival and pregnancy outcomes for human day 5 and 6 expanded blastocysts. Hum Reprod. 2004;19(12):2884–8.
57. Vanderzwalmen P, Bertin G, Debauche C, et al. Births after vitrification at morula and blastocyst stages: effect of artificial reduction of the blastocoelic cavity before vitrification. Hum Reprod. 2002;17(3):744–51.
58. Huang CC, Lee TH, Chen SU, et al. Successful pregnancy following blastocyst cryopreservation using super-cooling ultra-rapid vitrification. Hum Reprod. 2005;20(1):122–8.
59. Vanderzwalmen P, Bertin G, Debauche C, et al. Vitrification of human blastocysts with the Hemi-Straw carrier: application of assisted hatching after thawing. Hum Reprod. 2003;18(7):1504–11.
60. Schneider U, Mazur P. Osmotic consequences of cryoprotectant permeability and its relation to the survival of frozen-thawed embryos. Theriogenology. 1984;21(1):68–79.
61. Mukaida T, Oka C, Goto T, et al. Artificial shrinkage of blastocoeles using either a micro-needle or a laser pulse prior to the cooling steps of vitrification improves survival rate and pregnancy outcome of vitrified human blastocysts. Hum Reprod. 2006; 21(12):3246–52.
62. Richter KS, Shipley SK, McVearry I, et al. Cryopreserved embryo transfers suggest that endometrial receptivity may contribute to reduced success rates of later developing embryos. Fertil Steril. 2006;86(4):862–6.
63. Son WY, Yoon SH, Yoon HJ, et al. Pregnancy outcome following transfer of human blastocysts vitrified on electron microscopy grids after induced collapse of the blastocoele. Hum Reprod. 2003;18(1):137–9.
64. Iwayama H, Hochi S, Yamashita M. In vitro and in vivo viability of human blastocysts collapsed by laser pulse or osmotic shock prior to vitrification. J Assist Reprod Genet. 2011;28(4):355–61.
65. Hur YS, Park JH, Ryu EK, et al. Effect of artificial shrinkage on clinical outcome in fresh blastocyst transfer cycles. Clin Exp Reprod Med. 2011;38(2):87–92.
66. Shapiro BS, Richter KS, Harris DC, et al. A comparison of day 5 and day 6 blastocyst transfers. Fertil Steril. 2001;75(6):1126–30.
67. Levens ED, Whitcomb BW, Hennessy S, et al. Blastocyst development rate impacts outcome in cryopreserved blastocyst transfer cycles. Fertil Steril. 2008;90(6):2138–43.
68. Liebermann J, Tucker MJ. Vitrifying and warming of human oocytes, embryos, and blastocysts: vitrification procedures as an alternative to conventional cryopreservation. Methods Mol Biol. 2004;254:345–64.

Should We Eliminate Fresh Embryo Transfer from ART?

16

Daniel J. Kaser, Maria Assens, and Catherine Racowsky

16.1 Introduction

Since the first report of a human pregnancy following cryopreserved embryo transfer (CET) in 1983 [1], the efficiency of embryo cryopreservation has dramatically improved. Effective embryo storage allows the opportunity to salvage a cycle at risk for ovarian hyperstimulation syndrome (OHSS); it provides an option for fertility preservation; and it provides the additional time necessary for unsynchronized embryo donation and preimplantation genetic diagnosis of heritable diseases [2]. This technology also has the potential to reduce multiple gestation rates through an elective single ET paradigm in which embryos from the same stimulation are transferred, one at a time, in subsequent cycles.

Transfer of one or more fresh embryos remains the first-line approach for attempting to establish a pregnancy following IVF/ICSI. What has only recently become apparent, though, is that ongoing pregnancy rates are higher and obstetric and neonatal outcomes are possibly improved following CET compared with fresh embryo transfer [3]. By uncoupling the processes of ovarian stimulation and embryo replacement, any deleterious effects of COH on endometrial receptivity can be minimized. As a result not only are pregnancy rates increased, but also the incidence of certain adverse outcomes like ectopic pregnancy, ovarian hyperstimulation, preeclampsia, antepartum hemorrhage, low birth weight, and perinatal mortality may be reduced [3–9]. This chapter reviews the evidence supporting elective CET, the optimal developmental stage and preferred method for embryo cryopreservation, and the implications for large-scale adoption of this clinical practice.

16.2 Normal Physiology of Embryo Implantation

During the normal menstrual cycle, the human endometrium undergoes a series of developmental changes in the glandular, stromal, and vascular compartments in preparation for implantation of a viable embryo [10]. In response to rising levels of serum estradiol from the dominant follicle, glandular proliferation, pseudostratification, and ciliation occurs in the functionalis layer. The stroma of the basal layer becomes edematous and is infiltrated by inflammatory cells, and the spiral end arteries coalesce to form a capillary network that perfuses the proliferative endometrium. Following ovulation, progesterone from the corpus luteum effects the glandular dilation and tortuosity, along with

D.J. Kaser, M.D. • M. Assens, M.D.
Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

C. Racowsky, Ph.D. (✉)
Department of Obstetrics and Gynecology, Division of Reproductive Endocrinlogy and Infertility, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA
e-mail: cracowsky@partners.org

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_16,

hypercoiling and engorgement of spiral arteries, thereby inducing the morphological changes characteristic of secretory endometrium. A conserved suite of genes is activated 7–11 days after the LH surge (corresponding to cycle days 20–24) to modulate successful apposition, adhesion, and invasion of the trophoblast during the "window of implantation." There are a myriad of immunohistochemical, ultrastructural, and serologic markers of this period of receptivity, including calcitonin, cyclophilin, osteopontin, insulin-like growth factor II, leukemia inhibitory factor, MUC-1, HOXA10, cadherin-11, interleukin-15, and pinopodes, among others [11–13]. It is critical to note that these biomarkers are, in turn, regulated by steroid response elements such that uterine receptivity is directly affected by the specific hormonal milieu found at the time of implantation.

16.3 Effect of COH on Endometrial Receptivity

Both GnRH agonist and antagonist protocols result in advances in endometrial maturation according to the Noyes morphologic criteria [14–16]. Histologic dating, however, has limited clinical application in current practice, as there is considerable variability in the appearance of normal mid-secretory phase endometrium and the expression of key genes thought to regulate implantation is altered in the absence of architectural changes in the glandular and stromal compartments [17].

More recently, several groups have used DNA microarray analysis to compare the transcriptome of the prereceptive and receptive endometrium [18–22]. Only Haouzi and colleagues, however, have analyzed the alterations in gene expression profiles in paired samples of the early and mid-secretory phases of the same patients in both natural and stimulated cycles. These authors report that when the microarray signature of endometrial biopsies obtained from unstimulated cycles were compared with those following COH with a GnRH agonist, there were important differences in representative genes involved in cell cycle checkpoints (Fig. 16.1a). Specifically, genes encoding cyclins, cell division cycle (CDC) members, cyclin-dependent kinases (CDK), and members of the E2F family of transcription factors were significantly downregulated in simulated cycles. Furthermore, genes that were normally upregulated in natural cycles, including those involved in TGF-β signaling, complement and coagulation cascades and leukocyte migration, failed to be upregulated in stimulated cycles. Haouzi et al. postulate that these differences are the result of prolonged exposure to supraphysiologic steroid concentrations or an inappropriate dose of gonadotropins during COH [20].

Similar to GnRH agonist cycles, GnRH antagonists also affect the gene expression profile during the transition from the prereceptive to receptive endometrium but perhaps to a lesser degree [13]. Thirty-six percent of genes upregulated in natural cycles are likewise upregulated in antagonist cycles, compared to only 5 % in long agonist protocols (Fig. 16.1b, c). Moreover, only two genes involved in cell cycle progression have altered expression patterns following antagonist treatment. Haouzi et al. conclude that in comparison to GnRH agonists, antagonist treatment more closely approximates the receptivity in a natural cycle.

16.4 Methods to Minimize Embryo/Endometrial Asynchrony

Two approaches have evolved to address this artificial asynchrony between the embryo and the stimulated endometrium in COH (1) GnRH antagonist pituitary suppression with a GnRH agonist trigger and modified luteal phase support and (2) elective cryopreservation of all embryos with subsequent replacement in a natural or programmed cycle.

16.4.1 GnRH Agonist Triggering in Patients Cotreated with a GnRH Antagonist

The use of a GnRH agonist to trigger ovulation and final oocyte maturation induces a flare-up of FSH and LH that mimics the natural surge in

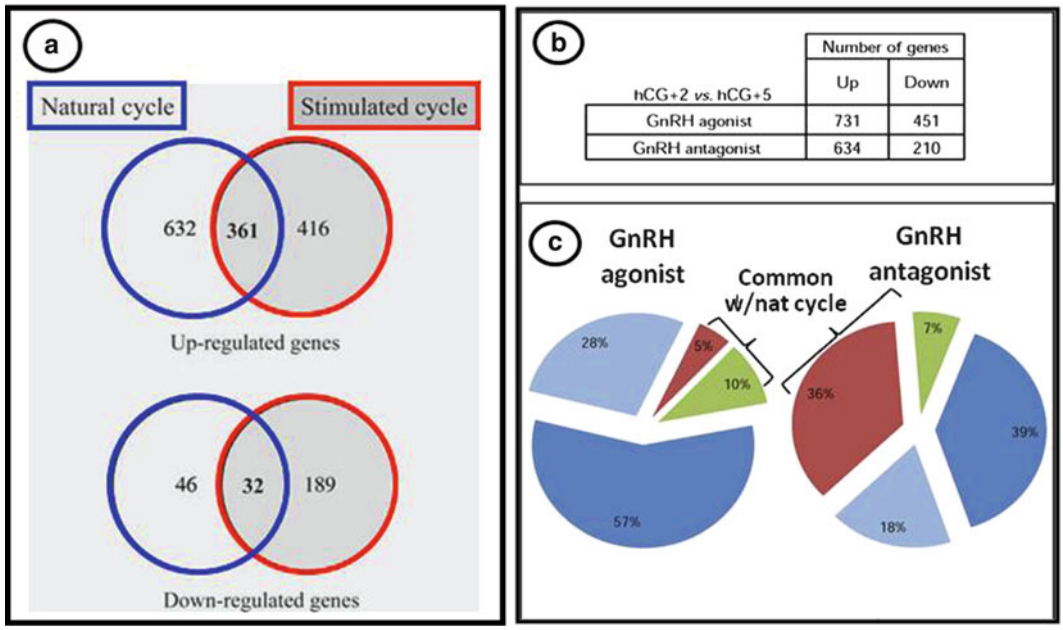

Fig. 16.1 Modulation of endometrial receptivity genes in natural and stimulated cycles. (*GnRH* gonadotropin-releasing hormone) (**a**) Venn diagram of up- and down-regulated endometrial receptivity genes in natural and GnRH-stimulated cycles. (**b**) Number of genes modulated in the early (hCG+2) and mid- (hCG+5) secretory endometrium in GnRH agonist and antagonist cycles. (Published with permission from Haouzi et al. [20].) (**c**) Percentage of genes up- or downregulated in GnRH agonist and antagonist cycles, along with the percentage in common with natural cycles. (*Blue*: upregulated genes exclusive to GnRH analogues; *light blue*: downregulated genes exclusive to GnRH analogues; *red*: upregulated genes in common with the natural cycle; *green*: downregulated genes in common with the natural cycle.) (Published with permission from Haouzi et al. [13])

gonadotropins in the late follicular phase [23, 24]. While the stimulated endogenous LH surge triggers ovulation, the FSH surge promotes nuclear maturation and LH receptor expression on the granulosa cells of the corpus luteum [25]. As a result of this additive FSH effect, several groups have demonstrated that more metaphase II oocytes are retrieved following a GnRH agonist trigger compared to a traditional hCG trigger, which only exerts an LH-like effect [25–28].

Early studies using a GnRH agonist trigger, however, showed extremely high rates of implantation failure and early pregnancy loss. In fact the first randomized controlled trial to assess clinical outcomes following a GnRH agonist trigger was stopped prematurely due to a 79 % early pregnancy loss rate despite standard luteal support [29]. If the method of luteal support was modified, though, with an additional bolus of 1,500 IU hCG on the day of oocyte retrieval to supplement a relative LH deficiency, clinical pregnancy rates were comparable to those following hCG trigger [26, 30].

These authors hypothesized that alterations in the expression of those genes involved in endometrial receptivity were responsible for these disparate results. By comparing the microarray signatures of endometrial biopsies obtained 5 days after oocyte retrieval in women cotreated with a GnRH antagonist for pituitary suppression and either a GnRH agonist trigger and 1,500 IU hCG for modified luteal support or a standard 10,000 IU hCG trigger and standard luteal support, Humaidan and colleagues demonstrated that endometrial gene expression in the mid-secretory phase can be normalized following GnRH agonist trigger with the addition of low-dose hCG at time of oocyte retrieval [31]. There were only two genes that were differentially expressed between these two groups, in comparison to 785 genes that were differentially expressed between cycles with and without modified luteal support.

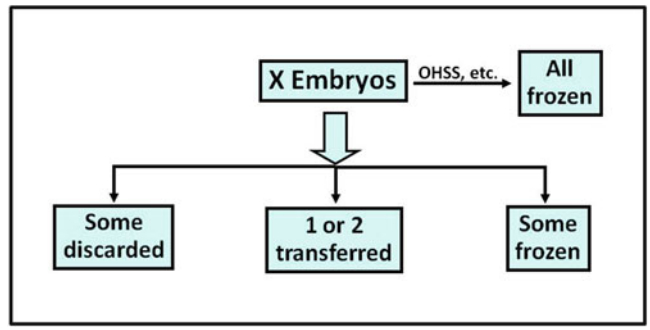

Fig. 16.2 Conventional paradigm for in vitro fertilization (*OHSS* ovarian hyperstimulation syndrome)

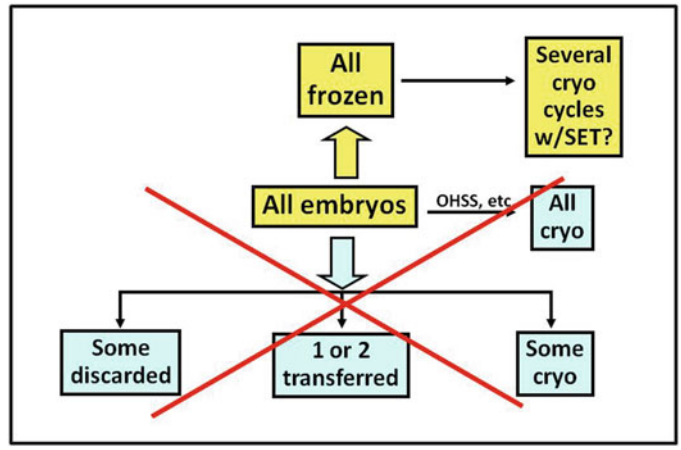

Fig. 16.3 Proposed paradigm for elective cryopreservation of all embryos with subsequent transfer (*OHSS* ovarian hyperstimulation syndrome, *SET* single embryo transfer)

16.4.2 Elective Cryopreservation of All Embryos with Subsequent Replacement

An alternative strategy to overcome the abnormal luteal phase in all stimulated cycles is to uncouple the processes of ovarian stimulation and embryo transfer; that is, by transferring cryopreserved embryos in a subsequent natural or programmed cycle, the asynchrony between embryo and endometrium can be mitigated. Indeed, the supraphysiologic steroid concentrations in a fresh cycle can be avoided altogether; the peak serum estradiol levels in natural or programmed cycles more closely resemble the hormonal environment in unassisted conception (103–526 pg/mL) [9]. As a result the above-described alterations in endometrial histology and transcriptional programs may be decreased.

In conventional practice, fresh embryos derived from COH are transferred to the uterus at the cleavage or blastocyst stage, and supernumerary embryos deemed suitable for subsequent transfer are then cryopreserved for later use. All embryos are frozen rarely in cases, for example, of risk of OHSS (Fig. 16.2). For elective CET, standard COH and IVF/ICSI insemination are performed, and all embryos are then cryopreserved and transferred at a later date (Fig. 16.3). There is no consensus regarding the stage of embryo to cryopreserve (pronucleate, cleavage, or blastocyst), the method of cryopreservation (slow-freeze vs. vitrification), the optimal time to wait between ovarian stimulation and subsequent ET, or the type of luteal support that should be prescribed. This approach of elective CET, and the evidence to support its clinical application, is the focus of the remainder of the chapter.

16.5 Clinical Outcomes Following Fresh ET Versus CET

16.5.1 Pregnancy Rates and Complications of IVF

As cryopreservation techniques have improved, pregnancy rates following CET have become similar to those from fresh cycles and may, in fact, now be superior. Indeed, three randomized studies have evaluated pregnancy rates in women allocated to elective CET or fresh transfer, the ongoing pregnancies of which are shown in Fig. 16.4a Aflatoonian and colleagues [32] randomized 374 high-responder patients less than age 38 to fresh ET on Day 3 or vitrification of Day 2 embryos with subsequent CET of Day 3 cleavage stage embryos. Luteal support was provided with 100 mg intramuscular progesterone daily until documentation of fetal cardiac activity or negative hCG. These authors reported that implantation rates and ongoing clinical pregnancy rates were higher following CET (implantation: 24.7 % vs. 17.5 %, $p<0.05$; ongoing pregnancy: 39.0 % vs. 27.8 %, OR 1.66, 95 % CI 1.07–2.56, $p=0.02$). Shapiro et al. [33] randomized 137 normal responders less than age 41 to fresh blastocyst ET or cryopreservation with slow-freeze at the pronuclear stage, followed by subsequent postthaw extended culture and transfer of Day 5 blastocysts. Intramuscular progesterone 100 mg daily was given for luteal support. Again, implantation and ongoing pregnancy

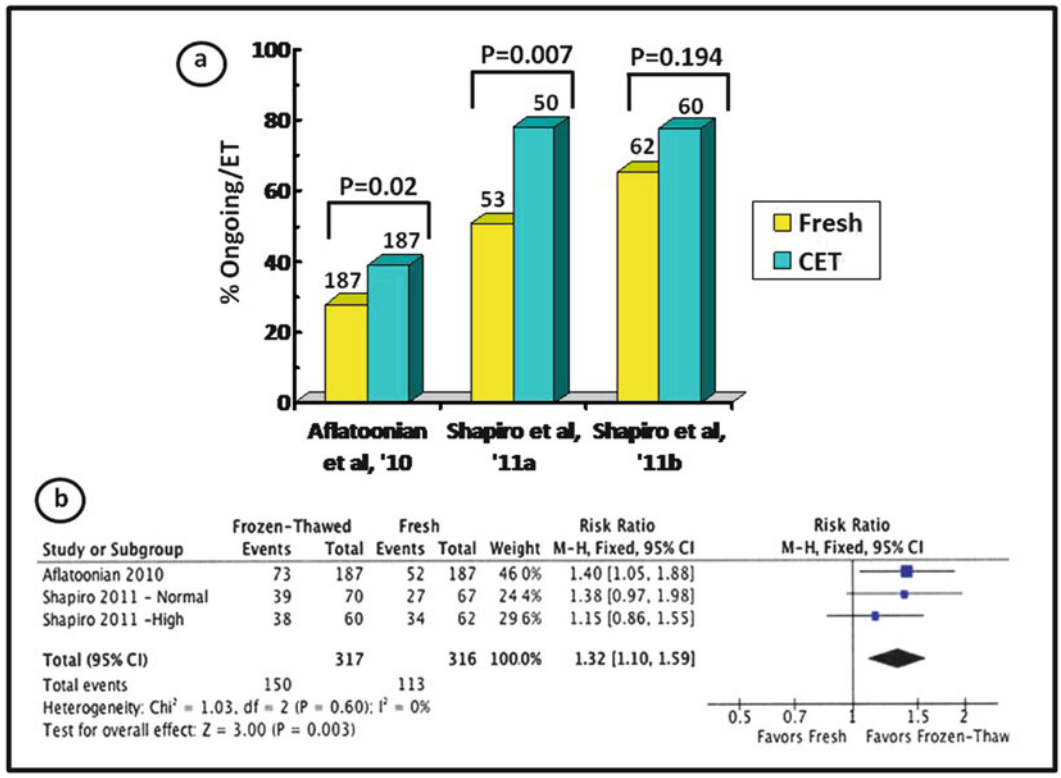

Fig. 16.4 Comparison of ongoing pregnancy rates in three randomized controlled trials of fresh vs. cryopreserved embryo transfer (*CET* cryopreserved embryo transfer); (**a**) Ongoing pregnancies in each study. (**b**) Meta-analysis results of ongoing pregnancy rates from fresh transfers compared with frozen–thaw transfers. Published with permission from Roque et al. [4]

rates were higher following CET (implantation: 70.8 % vs. 38.9 %, $p<0.0001$; ongoing pregnancy: 78.0 % vs. 50.9 %, $p<0.01$). In a parallel study of 122 high responders with the same experimental design, Shapiro and colleagues reported a similar effect size for ongoing pregnancies, though the higher pregnancy rates in the CET group did not reach statistical significance [34]. Importantly, none of these studies reported live birth rates. A meta-analysis of these three studies confirmed that patients allocated to CET had higher ongoing pregnancy rates compared with those who underwent fresh embryo transfer (RR 1.32, 95 % CI 1.10–1.59, $p=0.003$; Fig. 16.4b) [4].

In addition to higher implantation and clinical pregnancy rates in CET cycles, there is evidence to support a lower incidence of ectopic pregnancies and severe OHSS in freeze–thaw and freeze-all cycles [5, 6, 35].

16.5.2 Obstetric and Neonatal Outcomes

Several registry based cohort studies have evaluated neonatal outcomes after fresh as compared with CET cycles [3, 36, 37]. All report increased birth weights in the frozen–thawed transfer group, but no differences in perinatal mortality rates or rates of major congenital malformations. The observation of discrepant birth weights has been confirmed by several groups, including Kalra and colleagues, who published an analysis from the Society for Assisted Reproductive Technologies database of 38,626 singleton pregnancies conceived after fresh ET and 18,166 singletons conceived after CET [8]. These authors reported that the odds of overall low birth weight (10 % vs. 7.2 %; adjusted OR 1.35; 95 % CI 1.20–1.51), low birth weight at term (2.5 % vs. 1.2 %, adjusted OR 1.73, 95 % CI 1.31–2.29), and preterm low birth weight (34.1 % vs. 23.8 %, adjusted OR 1.49, 95 % CI 1.24–1.78) were all significantly higher after fresh ET, independent of both preterm birth and gestational age at delivery. Interestingly, there was no difference in the incidence of low birth weight infants in donor oocyte cycles following fresh ET or CET (11.5 % vs. 11.3 % adjusted OR 0.99, 95 % CI 0.82–1.18), in which the embryo recipient has not undergone COH.

A meta-analysis of 11 observational studies concluded that other obstetric and neonatal outcomes following delayed CET were superior to those from fresh ET. Specifically, in CET cycles, there were decreased rates of antepartum hemorrhage (RR = 0.67, 95 % CI 0.55–0.81), preterm birth (RR = 0.84, 95 % CI 0.78–0.90), small for gestational age (RR = 0.45, 95 % CI 0.30–0.66), low birth weight (RR = 0.69, 95 % CI 0.62–0.76), and perinatal mortality (RR = 0.68, 95 % CI 0.48–0.96) [38]. However, there were several limitations to this systematic review, with no adjustments made for potential confounders such as age, race, smoking, parity, prior preterm birth, or medical comorbidities. Furthermore, there was significant heterogeneity in study design and clinical protocols for COH, cryopreservation, endometrial preparation, and luteal support [7].

Finally, rates of preeclampsia (i.e., proteinuric hypertension) may be increased in fresh ET cycles due to supraphysiologic steroid concentrations. Imudia and colleagues reported that women with elevated serum estradiol levels on the Day of hCG (defined as >3,450 pg/mL, corresponding to the 90th percentile) and who underwent fresh ET, were more likely to develop preeclampsia than women who elected cryopreservation of all embryos with delayed ET (21.9 % vs. 0 %; likelihood ratio 7.47) [9]. The underlying reason for this difference is unknown but may be attributable to the supraphysiologic hormonal milieu at the time of implantation in fresh ET, which may impair trophoblast invasion and placental angiogenesis [39].

16.5.3 When and How to Freeze?

Despite a recent Alpha consensus meeting among embryologists to determine key performance indicators and benchmarks for embryo cryopreservation, no consensus exists regarding the optimal stage at which to cryopreserve embryos. Embryos can be frozen at the pronucleate stage on Day 1, the cleavage stage on Day 2 or 3, or the blastocyst stage on Days 5–7. There are advantages and disadvantages to each, as outlined below.

16.5.3.1 Cryopreservation at the Pronucleate, Cleavage, or Blastocyst Stage

By freezing zygotes on Day 1 at the pronucleate stage, there is no damage to the mitotic spindle or cytokinetic machinery, and postthaw viability is readily assessed by resumption of mitosis [40]. Some markers for developmental competency and embryo quality, such as proximity of pronuclei and the presence of cytoplasmic halos or nucleolar precursor bodies, may be affected by cryopreservation at this stage, which together may impact the cumulative pronuclear score [41, 42]. While some early studies reported that the implantation rate of cryopreserved pronucleate embryos exceeds that of cleavage stage embryos [43–45], the absence of prospective randomized trial data reduce the strength of this evidence.

Proponents of Day 2 or Day 3 freezing argue that culture until the cleavage stage allows selection of embryos that are most suitable for cryopreservation according to the morphologic criteria of cell number, symmetry, and fragmentation. Pregnancy rates following Day 3 cryopreservation have been shown to correlate with percent blastomere survival after thawing, lead cell number at transfer and resumption of mitosis in postthaw culture [46].

Cryopreservation at the blastocyst stage has the unique advantage of allowing trophectoderm biopsy for preimplantation genetic diagnosis which, with more cells biopsied compared with the one or two cells removed on Day 3, may improve accuracy of the testing. Moreover, despite challenges associated with the potential for crystal formation in the fluid-filled blastocoelic cavity, blastocysts may be less vulnerable to damage from the freeze–thaw process due to the selection pressure exerted by the extended culture necessary for their development [47, 48]. Blastocysts deemed appropriate for cryopreservation have a well-defined inner cell mass and adequate total cell number. The day of blastocyst expansion (Day 5 vs. Day 6 or 7) is predictive of pregnancy rates following freeze–thaw, with the highest rates for Day 5 blastocysts or Day 6 embryos following artificial collapse of the blastocoele [48]. Postthaw viability is ascertained by the extent of cellular degeneration; in one series, no embryos implanted when fewer than 80 % of cells survived the thaw process [49].

16.5.3.2 Method of Cryopreservation: Slow-Freeze Versus Vitrification

There are two types of cryopreservation that are routinely used in the ART laboratory: equilibrium cooling (also known as controlled slow-freezing) and vitrification (or ultra-rapid freezing). A one-step slow-freeze process was first described by Leibo [50], in which embryos suspended in a solution containing cryoprotectants such as dimethyl sulfoxide, glycerol, or 1,2-propanediol are cooled at a rate of ~0.4 °C/min to ~−6 °C, at which seeding is done with supercooled forceps to induce extracellular freezing and dehydration of the intracellular contents. After a postseeding hold time of ~15 min, the embryos are then cooled at a rate of ~0.4 °C down to a final temperature of approximately −40.0 °C before being plunged into liquid nitrogen for storage. Thawing is performed at a warming rate of approximately 250 °C/min.

Vitrification, first described by Rall and Fahy in 1985, refers to the reversible transition of liquid into an amorphous noncrystalline glass without the formation of ice crystals [51]. This method relies on high cryoprotectant concentrations, a cooling rate between 1,000 °C/min and 10,000 °C/min and very rapid warming. Early concerns regarding vitrification involved the use of highly concentrated cryoprotectants and their potential cytotoxic effects; however, as more clinical experience has accumulated, vitrification is now accepted as a safe alternative to slow-freezing [40].

Advantages of vitrification include reduced cryoinjury, less expensive equipment, increased postthaw survival, and a trend toward improved pregnancy rates [52–55]. Several studies support the preferential use of vitrification for pronuclear, cleavage, and blastocyst stage embryos [56–59]. Following thaw, 100 % of vitrified pronuclear embryos survived, compared to 89 % of pronuclear embryos subjected to slow-freezing [56]. A meta-analysis of four prospective studies with a total of 8,824 cryopreserved embryos (7,482 vitrified and 1,342 slow-frozen) demonstrated that vitrification resulted in a higher postthaw survival rate of both cleavage stage (97.5 % vs.

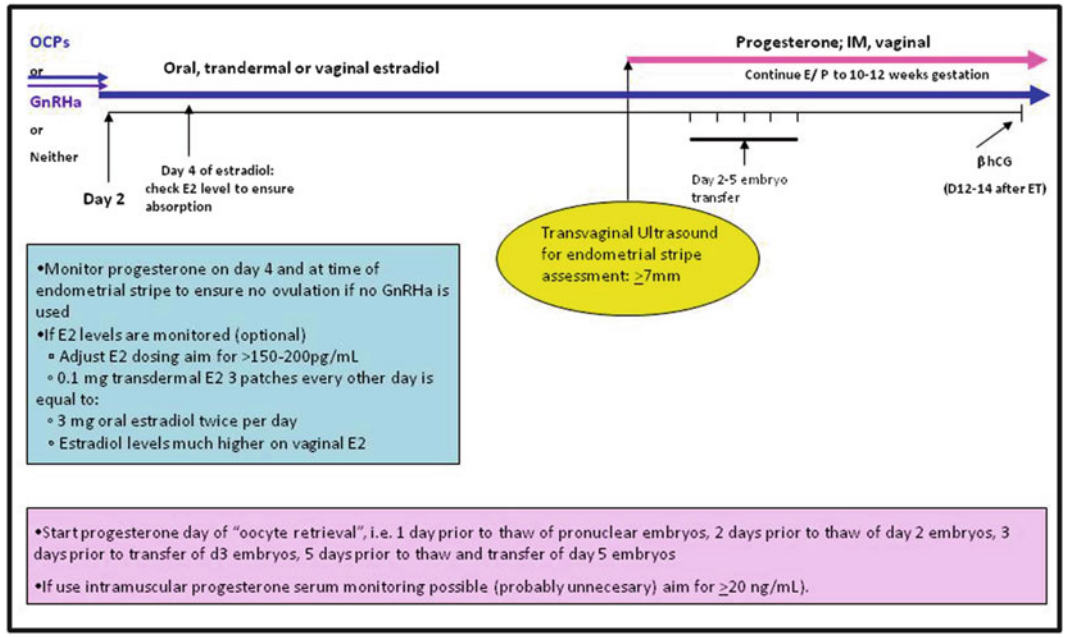

Fig. 16.5 One regimen for programmed cycle for replacement of cryopreserved embryos. Published with permission from VerMilyea et al. [40]

84.1 %, OR 15.57, 95 % CI 3.68–65.82; $p<0.001$) and blastocyst stage embryos (89.9 % vs. 75.4 %, OR 2.20, 95 % CI 1.53– 3.16; $p<0.0001$) [55]. Two of the four included studies compared implantation and ongoing pregnancy rates after transfer of vitrified or slow-frozen embryos. Rama Raju and colleagues [59] reported a higher ongoing pregnancy rate with vitrified cleavage stage embryos (35.0 % vs. 17.4 %), albeit this was not statistically significant. Kuwayama et al. [56] reported similar ongoing pregnancy rates for the two methods (53 % vs. 51 %). Thus, the weight of the evidence supports superior survival following vitrification of embryos at all stages. However, further more robust studies are required to determine whether clinical outcomes favor vitrification over slow-freezing techniques.

16.5.3.3 Regimens for Endometrial Preparation

There appears to be no difference between pregnancy rates in natural ovulatory CET cycles and those with artificial endometrial preparation. One series of 628 freeze–thaw cycles reported equivalent clinical pregnancy rates for natural and GnRH-agonist programmed cycles (28 % and 30 %, respectively) [60]. Live birth rates in another series with 1,677 CET cycles were also comparable (28.1 % vs. 27.8, respectively) [61]. It should be noted that anovulatory patients (e.g., those with polycystic ovarian syndrome) or those who require a flexible transfer date might benefit from a cycle with exogenous hormone, with or without pituitary suppression. Patients randomized to receive GnRH-agonist pretreatment ($n=53$) or not ($n=53$) also had pregnancy rates that were not statistically different (26.4 % and 21.1 %, respectively) [62]. Various hormone replacement regimens have been developed using different doses and routes of estrogen and progesterone, but there is no consensus regarding the most effective one [63]. One regimen is depicted in Fig. 16.5.

16.5.3.4 Types of Luteal Phase Support Following CET

As the corpus luteum does not develop in prepared cycles, pharmacologic luteal support is necessary. Options include intramuscular progesterone, which is considered the standard, along with intravaginal micronized formulations.

A recent meta-analysis concluded that there was insufficient evidence to recommend one method of luteal support over another in freeze–thaw and donor cycles [64]. Since then a multivariable analysis of Day 3 CET cycles comparing intramuscular progesterone to 8 % Crinone vaginal gel reported a higher live birth rate with the intramuscular route (39.1 % vs. 24.4 %, OR 0.51, 95 % CI 0.37–0.70, $p < 0.0001$ [65]. Those authors concluded that intramuscular progesterone should be the preferred route of administration until further research establishes its equivalence with 8 % Crinone vaginal gel.

16.6 Should Elimination of Fresh Transfer Be Universally Adopted?

Elimination of fresh ET in favor of elective cryopreservation of all embryos with subsequent replacement has the potential to improve the safety and efficacy of assisted reproductive technology. However, there is currently insufficient high-quality evidence to support its routine practice [7]. While available studies have demonstrated higher ongoing pregnancy rates and possibly fewer adverse obstetric and neonatal outcomes following elective cryopreservation, more research is needed to validate these preliminary findings. Until then, the current paradigm of COH with immediate fresh ET should continue to be the default approach, with elective CET being reserved for specific clinical indications (high risk of OHSS, PGD reporting issues, etc.) and, possibly, in select cases of repeat implantation failure.

16.7 Implications for Elective Cryopreservation of All Embryos

There are many unanswered questions about what a successful elective CET program would entail, including the following:

- Should all embryos be frozen, or only those deemed to be of "appropriate" quality?
- Would informed patients have the option for fresh vs. freeze–thaw cycles, and would this depend on the insurance mandate of particular states?
- What stage of embryo development is optimal for cryopreservation?
- Can slow-freeze protocols continue to be used in the face of better postthaw viability following vitrification?
- How can the process of vitrification be improved to ensure complete survival of all cryopreserved embryos?
- What is the minimum and preferred amount of time to wait between COH and subsequent CET?
- Are programmed cycles with exogenous hormone necessary?
- What is the most effective method for luteal phase support for cryopreserved cycles?
- Which clinical decision models for CET should be used to guide the number of cryopreserved embryos to transfer?

16.8 Conclusions

In order for an elective CET program to succeed on a large scale, the process of cryopreservation and thaw has to be safe, effective, and highly reproducible. Three randomized controlled trials demonstrate that elective cryopreservation of all embryos with subsequent embryo replacement is associated with increased implantation and ongoing pregnancy rates; however, none of these three trials reported live birth rate as an outcome. Several observational studies indicate that adverse outcomes such as ectopic pregnancy, ovarian hyperstimulation, preeclampsia, antepartum hemorrhage, low birth weight and perinatal mortality are reduced by uncoupling the processes of COH and ET, but the quality of evidence to support these findings is only moderate. With regard to cryopreservation technique, the optimal developmental stage for embryo freezing is a matter of active debate, with the caveat that extending culture to the blastocyst stage in good prognosis patients allows trophectoderm biopsy for preimplantation genetic diagnosis and aneuploidy screening. Available evidence in the field of embryo cryopreservation supports the preferential use of vitrification over slow-freezing, as

postthaw viability is superior for all stages of development (pronucleate, cleavage, and blastocyst). Pregnancy rates following CET in a natural cycle seem equivalent to those in a programmed cycle with or without GnRH agonist pretreatment and hormone replacement. Practical considerations, such as how patients would accept the paradigm of elective cryopreservation of all embryos, how laboratory work-flow and storage capacity would be changed by the increased demand for cryopreservation, and how third-party payers would compensate clinics for this segmented protocol, remain to be determined. Taken together, despite available data supporting improved pregnancy rates and obstetrical and neonatal outcomes from CET cycles, additional findings from robust adequately powered prospective trials are needed before we eliminate fresh embryo transfer from ART.

References

1. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature. 1983;305(5936):707–9.
2. Oehninger S, Mayer J, Muasher S. Impact of different clinical variables on pregnancy outcome following embryo cryopreservation. Mol Cell Endocrinol. 2000;169(1–2):73–7.
3. Pinborg A, Loft A, Aaris Henningsen AK, et al. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995–2006. Fertil Steril. 2010;94(4):1320–7.
4. Roque M, Lattes K, Serra S, et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. Fertil Steril. 2013;99(1):156–62.
5. Shapiro BS, Daneshmand ST, De Leon L, et al. Frozen-thawed embryo transfer is associated with a significantly reduced incidence of ectopic pregnancy. Fertil Steril. 2012;98(6):1490–4.
6. D'Angelo A. Ovarian hyperstimulation syndrome prevention strategies: cryopreservation of all embryos. Semin Reprod Med. 2010;28(6):513–8.
7. Maheshwari A, Bhattacharya S. Elective frozen replacement cycles for all: ready for prime time? Hum Reprod. 2013;28(1):6–9.
8. Kalra SK, Ratcliffe SJ, Coutifaris C, et al. Ovarian stimulation and low birth weight in newborns conceived through in vitro fertilization. Obstet Gynecol. 2011;118(4):863–71.
9. Imudia AN, Awonuga AO, Kaimal AJ, et al. Elective cryopreservation of all embryos with subsequent cryothaw embryo transfer in patients at risk for ovarian hyperstimulation syndrome reduces the risk of adverse obstetric outcomes: a preliminary study. Fertil Steril. 2013;99(1):168–73.
10. Speroff L, Fritz MA, editors. The histologic changes in the endometrium during an ovulatory cycle. 7th ed. Philadelphia, PA: Lippincott Williams & Williams; 2005.
11. Lessey BA. The use of biomarkers for the assessment of uterine receptivity. In: Gardner DK, Weissman A, Howles CM, Shohan Z, editors. Assisted reproductive techniques: laboratory and clinical perspectives. London: Martin Dunitz Ltd.; 2001. p. 353–66.
12. Usadi RS, Groll JM, Lessey BA, et al. Endometrial development and function in experimentally induced luteal phase deficiency. J Clin Endocrinol Metab. 2008;93(10):4058–64.
13. Haouzi D, Assou S, Dechanet C, et al. Controlled ovarian hyperstimulation for in vitro fertilization alters endometrial receptivity in humans: protocol effects. Biol Reprod. 2010;82(4):679–86.
14. Barash A, Czernobilsky B, Insler V, et al. Endometrial morphology and hormonal profiles in in vitro fertilization patients. Eur J Obstet Gynecol Reprod Biol. 1992;44(2):117–21.
15. Kolibianakis E, Bourgain C, Albano C, et al. Effect of ovarian stimulation with recombinant follicle-stimulating hormone, gonadotropin releasing hormone antagonists, and human chorionic gonadotropin on endometrial maturation on the day of oocyte pick-up. Fertil Steril. 2002;78(5):1025–9.
16. Saadat P, Boostanfar R, Slater CC, et al. Accelerated endometrial maturation in the luteal phase of cycles utilizing controlled ovarian hyperstimulation: impact of gonadotropin-releasing hormone agonists versus antagonists. Fertil Steril. 2004;82(1):167–71.
17. Young SL, Lessey B, Balthazar U, et al. Defining the relationship between progesterone dose, endometrial histology and gene expression using an in vivo luteal phase defect model. Reprod Sci. 2011;18:273A.
18. Carson DD, Lagow E, Thathiah A, et al. Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening. Mol Hum Reprod. 2002;8(9):871–9.
19. Diaz-Gimeno P, Horcajadas JA, Martinez-Conejero JA, et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. Fertil Steril. 2011;95(1):50–60. e1-15.
20. Haouzi D, Assou S, Mahmoud K, et al. Gene expression profile of human endometrial receptivity: comparison between natural and stimulated cycles for the same patients. Hum Reprod. 2009;24(6):1436–45.
21. Mirkin S, Arslan M, Churikov D, et al. In search of candidate genes critically expressed in the human endometrium during the window of implantation. Hum Reprod. 2005;20(8):2104–17.
22. Talbi S, Hamilton AE, Vo KC, et al. Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological

processes in normo-ovulatory women. Endocrinology. 2006;147(3):1097–121.
23. Gonen Y, Balakier H, Powell W, et al. Use of gonadotropin-releasing hormone agonist to trigger follicular maturation for in vitro fertilization. J Clin Endocrinol Metab. 1990;71(4):918–22.
24. Itskovitz J, Boldes R, Levron J, et al. Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing hormone agonist. Fertil Steril. 1991;56(2):213–20.
25. Humaidan P, Bungum L, Bungum M, et al. Reproductive outcome using a GnRH antagonist (cetrorelix) for luteolysis and follicular synchronization in poor responder IVF/ICSI patients treated with a flexible GnRH antagonist protocol. Reprod Biomed Online. 2005;11(6):679–84.
26. Humaidan P, Papanikolaou EG, Tarlatzis BC. Reply: GnRHa to trigger final oocyte maturation: a time to reconsider. Hum Reprod. 2010;25(3):807–8.
27. Humaidan P, Kol S, Papanikolaou EG. GnRH agonist for triggering of final oocyte maturation: time for a change of practice? Hum Reprod Update. 2011;17(4):510–24.
28. Oktay K, Turkcuoglu I, Rodriguez-Wallberg KA. GnRH agonist trigger for women with breast cancer undergoing fertility preservation by aromatase inhibitor/FSH stimulation. Reprod Biomed Online. 2010;20(6):783–8.
29. Humaidan P, Bredkjaer HE, Bungum L, et al. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study. Hum Reprod. 2005;20(5):1213–20.
30. Humaidan P, Bungum L, Bungum M, et al. Rescue of corpus luteum function with peri-ovulatory HCG supplementation in IVF/ICSI GnRH antagonist cycles in which ovulation was triggered with a GnRH agonist: a pilot study. Reprod Biomed Online. 2006;13(2):173–8.
31. Humaidan P, Papanikolaou EG, Kyrou D, et al. The luteal phase after GnRH-agonist triggering of ovulation: present and future perspectives. Reprod Biomed Online. 2012;24(2):134–41.
32. Aflatoonian A, Oskouian H, Ahmadi S, et al. Can fresh embryo transfers be replaced by cryopreserved-thawed embryo transfers in assisted reproductive cycles? A randomized controlled trial. J Assist Reprod Genet. 2010;27(7):357–63.
33. Shapiro BS, Daneshmand ST, Garner FC, et al. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. Fertil Steril. 2011;96(2):344–8.
34. Shapiro BS, Daneshmand ST, Garner FC, et al. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfers in high responders. Fertil Steril. 2011;96(2):516–8.
35. Ishihara O, Kuwahara A, Saitoh H. Frozen-thawed blastocyst transfer reduces ectopic pregnancy risk: an analysis of single embryo transfer cycles in Japan. Fertil Steril. 2011;95(6):1966–9.
36. Wennerholm UB, Hamberger L, Nilsson L, et al. Obstetric and perinatal outcome of children conceived from cryopreserved embryos. Hum Reprod. 1997;12(8):1819–25.
37. Pelkonen S, Koivunen R, Gissler M, et al. Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995–2006. Hum Reprod. 2010;25(4):914–23.
38. Maheshwari A, Pandey S, Shetty A, et al. Obstetric and perinatal outcomes insingleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril. 2012;98:368–77.
39. Albrecht ED, Bonagura TW, Burleigh DW, et al. Suppression of extravillous trophoblast invasion of uterine spiral arteries by estrogen during early baboon pregnancy. Placenta. 2006;27(4–5):483–90.
40. VerMilyea MD, Liebermann J, Tucker MJ. Embryo cryopreservation. In: Ginsburg ES, Racowsky C, editors. In vitro fertilization: a comprehensive guide. New York: Springer; 2012. p. 145–60.
41. Scott L. Pronuclear scoring as a predictor of embryo development. Reprod Biomed Online. 2003;6(2):201–14.
42. Senn A, Urner F, Chanson A, et al. Morphological scoring of human pronuclear zygotes for prediction of pregnancy outcome. Hum Reprod. 2006;21(1):234–9.
43. Veeck LL, Amundson CH, Brothman LJ, et al. Significantly enhanced pregnancy rates per cycle through cryopreservation and thaw of pronuclear stage oocytes. Fertil Steril. 1993;59(6):1202–7.
44. Horne G, Critchlow JD, Newman MC, et al. A prospective evaluation of cryopreservation strategies in a two-embryo transfer programme. Hum Reprod. 1997;12(3):542–7.
45. Senn A, Vozzi C, Chanson A, et al. Prospective randomized study of two cryopreservation policies avoiding embryo selection: the pronucleate stage leads to a higher cumulative delivery rate than the early cleavage stage. Fertil Steril. 2000;74(5):946–52.
46. Sole M, Santalo J, Rodriguez I, et al. Correlation between embryological factors and pregnancy rate: development of an embryo score in a cryopreservation programme. J Assist Reprod Genet. 2011;28(2):129–36.
47. Veeck LL, Bodine R, Clarke RN, et al. High pregnancy rates can be achieved after freezing and thawing human blastocysts. Fertil Steril. 2004;82(5):1418–27.
48. Liebermann J, Tucker MJ. Comparison of vitrification and conventional cryopreservation of day 5 and day 6 blastocysts during clinical application. Fertil Steril. 2006;86(1):20–6.
49. Shipley SK, Richter KS, Tucker MJ, et al. Single embryo transfer of frozen-thawed blastocysts:

proportion of cells surviving the cryopreservation process is indicative of implantation potential. Fertil Steril. 2006;86:S332.
50. Leibo SP. A one-step method for direct nonsurgical transfer of frozen-thawed bovine embryos. Theriogenology. 1984;21(5):767–90.
51. Rall WF, Fahy GM. Ice-free cryopreservation of mouse embryos at -196 degrees C by vitrification. Nature. 1985;313(6003):573–5.
52. Kuleshova LL, Lopata A. Vitrification can be more favorable than slow cooling. Fertil Steril. 2002; 78(3):449–54.
53. Vajta G, Nagy ZP. Are programmable freezers still needed in the embryo laboratory? Review on vitrification. Reprod Biomed Online. 2006;12(6): 779–96.
54. Balaban B, Urman B, Ata B, et al. A randomized controlled study of human Day 3 embryo cryopreservation by slow freezing or vitrification: vitrification is associated with higher survival, metabolism and blastocyst formation. Hum Reprod. 2008;23(9): 1976–82.
55. Loutradi KE, Kolibianakis EM, Venetis CA, et al. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. Fertil Steril. 2008;90(1):186–93.
56. Kuwayama M, Vajta G, Kato O, et al. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod Biomed Online. 2005;11(3):300–8.
57. Huang CC, Lee TH, Chen SU, et al. Successful pregnancy following blastocyst cryopreservation using super-cooling ultra-rapid vitrification. Hum Reprod. 2005;20(1):122–8.
58. Zheng WT, Zhuang GL, Zhou CQ, et al. Comparison of the survival of human biopsied embryos after cryopreservation with four different methods using non-transferable embryos. Hum Reprod. 2005;20(6): 1615–8.
59. Rama Raju GA, Haranath GB, Krishna KM, et al. Vitrification of human 8-cell embryos, a modified protocol for better pregnancy rates. Reprod Biomed Online. 2005;11(4):434–7.
60. Queenan Jr JT, Veeck LL, Seltman HJ, et al. Transfer of cryopreserved-thawed pre-embryos in a natural cycle or a programmed cycle with exogenous hormonal replacement yields similar pregnancy results. Fertil Steril. 1994;62(3):545–50.
61. Givens I. Animal nutrition and lipids in animal products and their contribution to human intake and health. Nutrients. 2009;1(1):71–82.
62. Simon A, Hurwitz A, Zentner BS, et al. Transfer of frozen-thawed embryos in artificially prepared cycles with and without prior gonadotrophin-releasing hormone agonist suppression: a prospective randomized study. Hum Reprod. 1998;13(1O):2712–7.
63. Younis JS, Simon A, Laufer N. Endometrial preparation: lessons from oocyte donation. Fertil Steril. 1996;66(6):873–84.
64. Glujovsky D, Pesce R, Fiszbajn G, et al. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. Cochrane Database Syst Rev. 2010;1:CD006359.
65. Kaser DJ, Ginsburg ES, Missmer SA, et al. Intramuscular progesterone versus 8 % Crinone vaginal gel for luteal phase support for day 3 cryopreserved embryo transfer. Fertil Steril. 2012;98(6):1464–9.

ICSI Is a Revolutionary Treatment of Male Infertility That Should Be Employed Discriminately and Further Studied

17

Douglas T. Carrell

17.1 Introduction

The injection of sperm into oocytes has a long history and was initially undertaken as a means to study fertilization in model organisms. The first known report of sperm injection occurred in the sea urchin, not surprisingly, because of their size and use as an early model organism for developmental biology studies [1]. Soon after, microinjection was performed in mammals and ultimately resulted in a live birth in rabbits in 1988 [2, 3]. Despite technological advancements during that period, microinjection success rates remained quite low due to damage to the oocyte during the microinjection and after placement of the live sperm into the cytoplasm. However, the utility of using microinjection as a means to study sperm chromatin decondensation and pronuclear development was clearly apparent and invaluable in descriptive studies of fertilization.

D.T. Carrell, Ph.D, H.C.L.D. (✉)
Andrology and IVF Laboratories, Division of Urology, Department of Surgery, University of Utah School of Medicine,, 675 Arapeen Dr. Suite 205, Salt Lake City, UT 84108, USA

Department of Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, UT 84108, USA

Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT 84108, USA
e-mail: douglas.carrell@hsc.utah.edu

The development of the use of microinjection as a means to treat human infertility was indirect and gradual. In 1988, Lanzendorf et al. published two studies utilizing the microinjection of human sperm. In their first report, they demonstrated that round-headed sperm injected into human oocytes were able to undergo chromatin decondensation [4]. In the second report, the possible use of microinjection as a test system to assess normal pronuclear formation potential was studied in a few patients [5]. The reports by Lanzendorf et al. helped lay a foundation for microinjection of sperm as a therapeutic option, but it is critical to remember that other micromanipulation techniques were already undergoing extensive evaluation and use in patients undergoing in vitro fertilization, including zona-drilling, partial zona dissection (PZD) and subzonal sperm injection (SUZI) [6]. While early micromanipulation techniques were an advancement because patients with severe oligozoospermia could be treated, the actual success rates were quite modest and still required an adequate number of motile sperm, while the incidence of polyspermy was quite high [6, 7].

In 1992, Palermo et al. reported the birth of the first baby conceived by ICSI [8]. Palermo has recounted that he actually was in the process of performing SUZI when one sperm "accidentally" entered the cytoplasm when the oolemma was lysed [9]. This oocyte went on to fertilize and resulted in a viable offspring. Almost immediately, the potential benefits of ICSI were realized and subsequent reports validated improved outcomes in an expanding range of patient's etiologies [10].

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_17, © Springer Science+Business Media New York 2013

The European Society of Human Reproduction and Embryology (ESHRE) recently estimated that more than five million babies have been born as the result of ART, and that globally more than 1.5 million ART cases are performed annually resulting in approximately 350,000 babies per year [11]. Data from the Center for Disease Control and Prevention (CDC) clearly indicate that the percentage of ART cycles employing ICSI is >66 %, and that the trend is increasing [12]. These statistics suggest that ICSI may be increasing in usage for many patients undergoing IVF for reasons other than male factor infertility, since the percentage of men with severe oligozoospermia does not seem to be increasing in a manner mirroring the increased usage of ICSI. Anecdotally, there are reports of employing ICSI for cases as diverse as advanced maternal age, low numbers of oocytes, and in some cases, across the board for all ART patients.

The aim of this chapter is to suggest that ICSI should be used discriminately. The underlying question is not if ICSI is beneficial to many infertile couples, but rather if ICSI is being used unnecessarily and without justification in some patients, and what risks (physical and/or financial) may be increased to patients and offspring when unjustified ICSI is performed.

17.2 Risk to ICSI Offspring

There is no doubt that ICSI has resulted in the birth of millions of healthy babies that otherwise could not have been conceived. Has the technique, however, increased the risk of anomalies in the offspring? This question is relevant, but a thorough review of the data is beyond the scope or objective of this chapter. However, several recent studies have evaluated the risk of low birth weight and malformation rates in offspring conceived by ICSI compared to standard IVF or spontaneous conceptions from fertile controls. Due to the difficulty of properly accounting for confounding factors such as multiple births, parent's age, fertility status, etc., and the difficulty of studying a proper control group, it has been extremely difficult to reach a consensus on the data, and most of the studies have been met with skepticism.

Two important factors are relevant in considering the data from studies evaluating ICSI outcome. First, although the conclusions of the various studies are mixed, it does appear that ICSI may be associated with a slight-to-moderate increase in risk to the offspring [13–17]. However, the question of whether the elevated risk is due to the procedure itself or if the risk is associated with the patient population (infertile men) has not been definitively answered. It is clear that men with oligozoospermia, nonobstructive azoospermia, and other severe forms of male factor infertility possess an elevated risk of sperm chromosome aneuploidy, translocations, and/or DNA damage [18–21]. Interestingly, it has also been shown that oligozoospermic men carry a higher frequency of minor-allelle polymorphisms throughout their genome, altered DNA methylation, and increased genetic instability [22–24]. These studies clearly highlight the potential for elevated risk of genetic anomalies in offspring conceived from this population of patients undergoing ICSI. Unfortunately, no studies have yet specifically studied offspring conceived from ICSI with sperm from normozoospermic men (e.g., semen donors). Given the increasing use of ICSI, such a study should be possible.

Nevertheless, careful analysis of the data from some large outcome studies has highlighted a possible "procedure" component to the discussion of the risk from ICSI. For example, Davies et al. recently reported on the risk of ART to offspring in a large study from Australia and reported an unadjusted odds ratio of birth defects of 1.72 and an adjusted odds ratio of 1.55 for ICSI compared to spontaneous conceptions. Interestingly, spontaneous conceptions in patients who had previously undergone ART yielded an odds ratio of 1.27 (1.26 adjusted odds ratio), significantly lower than those undergoing ART with ICSI. While the data in this study and essentially all reports are not perfect, it is interesting to note that generally the data do indicate a slightly elevated risk, and no studies have reported a decreased risk [25].

Major malformations or diseases demonstrated in the first few years of life may not be representative of the greatest risk to ICSI offspring. The risk of many diseases, such as cardiovascular disease, cancer, and diabetes, may not

be demonstrated until later in life [26]. This may be particularly relevant in diseases that are the result of epigenetic variation, a field that is just emerging and appears to be critical to health. Epigenetic changes to an individual are modifications in the methylation of DNA or chemical changes to the proteins that bind DNA that alter gene expression [27]. An individual's "epigenome" is the genome-wide sum of all the chemical modifications and it is believed to be the link between the environment and its affect on many diseases [28]. A very critical factor in assessing the potential risk of ICSI to long-term health of the offspring is evaluating if ICSI may put individuals at an increased risk of epigenetic abnormalities due differences in the fertilization process itself.

17.3 Postfertilization and Early Embryonic Differences in ICSI-Derived Embryos

Despite the very large number of births resulting from ICSI, very few studies have actually studied the fertilization process after ICSI, and differences in human and rodent sperm ultrastructure and ICSI techniques reduce the utility of such studies in animal studies. Hewitson et al. performed a series of elegant ultrastructural study of ICSI using the rhesus monkey model, which is similar to humans in both ultrastructure and the fact that the centrosome is inherited from the sperm [29–31]. In their study, Hewitson et al. highlighted several differences in fertilization events following ICSI as compared to standard fertilization, including differences in the activation of the oocyte and aster formation, asynchronous decondensation of the sperm chromatin, differences in the fate of sperm tail components, differences in localization of acrosomal enzymes, and differences in the process of DNA synthesis [31]. The observation by Hewitson et al. reported that ICSI resulted in delayed DNA synthesis in the nonhuman primate model has also been confirmed in the mouse model [31, 32].

Figure 17.1 clearly demonstrates the asynchronous decondensation of sperm chromatin that was present regardless of whether acrosome intact or acrosome-reacted sperm were injected (Fig. 17.1). Abnormal sperm DNA decondensation is of interest for two major reasons, first, it has been shown that the sex chromosomes are generally located within the apical region of the sperm head, and delayed decondensation may theoretically result in an increased risk of chromosome aneuploidy, and event that has been shown to be elevated in patients undergoing ICSI [33]. Second, it has been clearly shown that the sperm chromatin normally has a very unique epigenetic programming that suggests a role for the sperm genome in assisting in early embryonic events [34]. Additionally, it is well known that chromatin undergoes specific and important epigenetic remodeling events during the early pronuclear stage [35]. Therefore, it is of potential relevance and concern if decondensation of the paternal chromatin is in any way delayed or altered, since such alterations may, theoretically, alter early embryonic gene expression.

Giritharan et al. have specifically looked at gene transcription following ICSI in a mouse model and found broad and significant differences in gene expression of embryos derived from ICSI, in fact about 1,000 genes had significantly different gene expression in blastocysts derived from ICSI compared to standard insemination [36]. Interestingly, they also evaluated gene expression abnormalities from embryos derived from culture in suboptimal culture medium and found far fewer gene expression differences (41 genes) existed in embryos cultured in suboptimal medium than in embryos derived from ICSI that were cultured in the control (good) culture medium. Gene expression abnormalities included both overexpression and underexpression of specific genes (Fig. 17.2). These data are supported by Bridges et al., who also reported differences in gene expression in blastocysts following ICSI, primarily in genes related to development [37]. Lastly, Kohda et al. evaluated gene expression in mice derived from standard IVF and ICSI/IVF and found that gene expression differences were retained in neonates of ICSI-derived pups but not from standard IVF pups (Fig. 17.3) [38]. Importantly, Kohda et al. saw no differences in the gene expression profiles at the adult stage. Nevertheless, these studies clearly

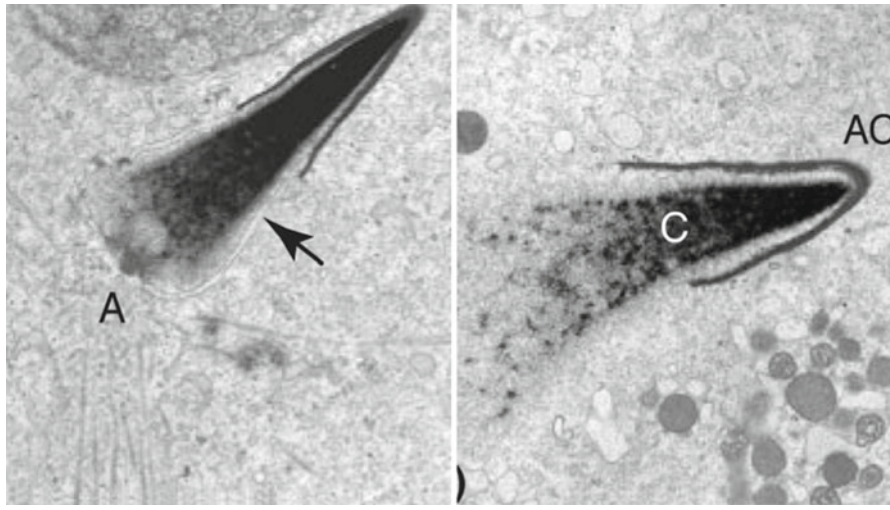

Fig. 17.1 Electron micrographic imaging demonstrates nonsynchronous decondensation of the sperm chromatin following ICSI in the nonhuman primate model (rhesus monkeys). In the *left panel*, the intact acrosome membrane is observed and chromatin decondensation has begun in the basal region but not the apical region. An aster (A) of microtubules can be observed. In the *right panel*, basal chromatin decondensation has progressed, but the apical region lags behind in decondensation of the chromatin (C) (from Hewitson et al., with permission [31])

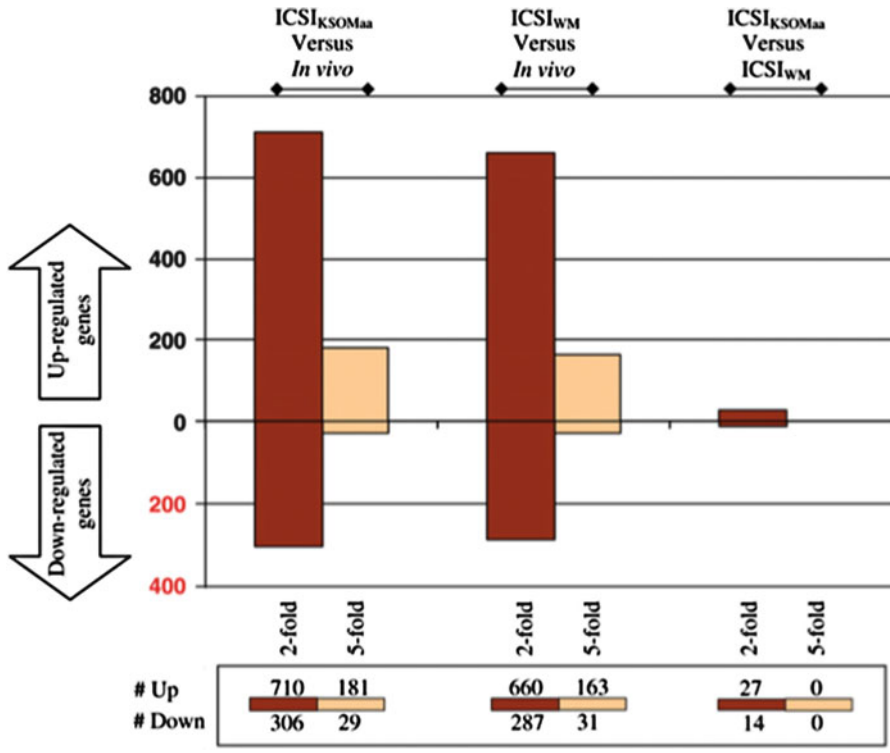

Fig. 17.2 Upregulation and downregulation of gene expression from blastocysts compared between two treatment options. Embryos were compared between ICSI and culture in optimal medium (KSOMaa) and in vivo derived embryos (*left graph*), ICSI and culture in suboptimal medium (Whitten's medium, WM, center graph), and ICSI followed by culture in either of the two culture media (*right graph*). The graphs demonstrate significant differences of upregulation and downregulation of genes when ICSI is employed, regardless of the medium used for culture (from Giritharan et al., with permission [36])

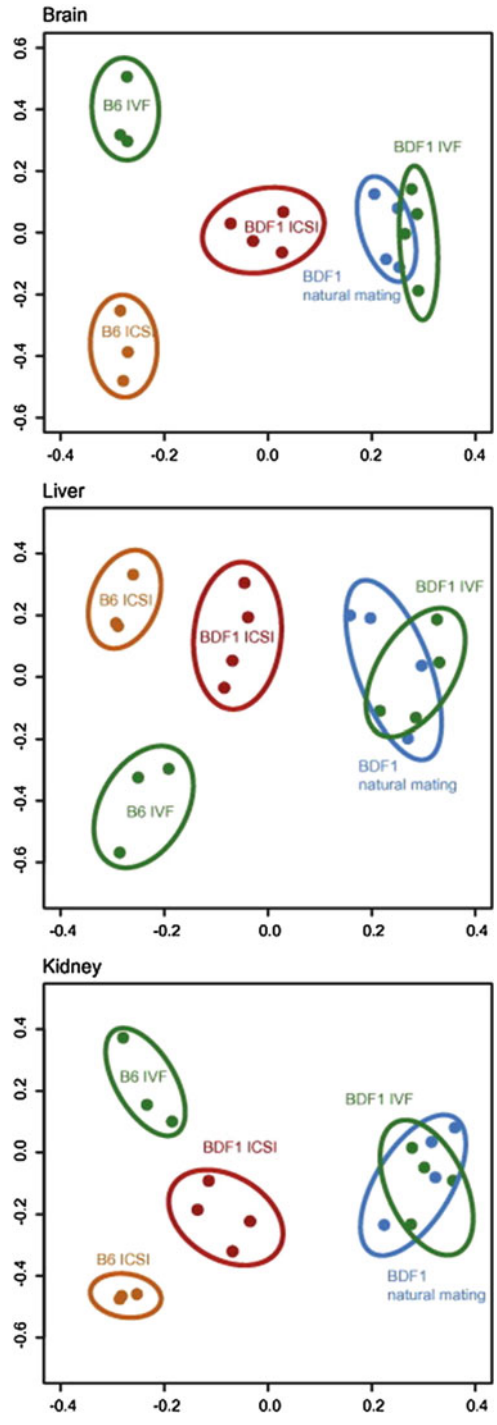

Fig. 17.3 Principle component analysis (PCA) of gene expression in three tissues of mouse neonates derived from ICSI compared to pups derived from IVF or natural mating. In all three tissues, differences close similarity is seen in gene expression clusters for natural mating and IVF in IVF with BDF1 medium. However, gene expression clusters very differently in pups derived from ICSI and BDF1 medium, demonstrating a strong effect of the procedure (from Kohda T, with permission [38])

demonstrate differences in gene transcription in embryos and neonates following ICSI that may have the potential of translating to real biological differences in the offspring.

Another difference in embryos derived from ICSI is a decreased number of inner cell mass (ICM) blastomeres compared to controls. This observation was reported in two of the studies described above [36, 37]. Given the key role of the inner cell mass in fetal development, this observation is of keen interest. Equally important, however, Giritharan et al. reported an even stronger effect of ICSI on the number of trophectoderm cells [36]. A recent study by Hill et al. reported that trophectoderm quality, including the number of cells presents, correlated with implantation and live birth rate in patients undergoing IVF [39]. These data demonstrated another area of concern in considering differences between ICSI-derived embryos and control embryos.

The differences described above clearly demonstrate that embryos derived from ICSI are different from embryos derived from standard IVF. While the data do not clearly demonstrate such differences lead to an increased risk of disease or anomalies in the offspring, it is important to remember that the risk for many diseases usually expressed in later life has not yet been evaluated in ICSI offspring. Therefore, differences in gene expression, epigenetic marks, or other emerging areas of study must be monitored and further studied before safety can be assured.

17.4 Fallacies Leading to Increased Usage of ICSI

Unnecessary usage of ICSI has been accelerated by certain fallacies purporting advantages from ICSI that are unsubstantiated by the data. Included amongst these fallacies are the beliefs that ICSI is advantageous in cases of low numbers of oocytes, that ICSI helps avoid fertilization failure and failure to have embryos available for transfer, and that ICSI is financially advantageous to the patient. In reality, the data available do not support these suppositions.

It is interesting to note that according to data reported in the Center for Disease Control and Prevention (CDC) 1999–2008 report, for each

year of the study, pregnancy rates were consistently higher for cases without ICSI compared to cycles with ICSI (Fig. 17.3) [12]. This was true whether donor sperm was used or nondonor sperm was used. Since donor sperm are by definition normozoospermic (most sperm banks accept donors only if their semen quality is in the top 10–20 percentile of quality), these data are particularly valuable in assessing the inherent benefit, or lack of benefit, of performing ICSI on a routine basis. Not only was no improvement observed, but also the pregnancy rate consistently trended below the rate for patients using donor sperm without ICSI. Similarly, the pregnancy rate was consistently lower in patients using homologous sperm with ICSI, compared to those not using ICSI, despite the fact that severe male factor patients usually present for IVF at a younger age than nonmale factor patients.

According to data published by the Human Fertilisation and Embryology Authority (HFEA) in Great Britain, ICSI outcomes are slightly, but not significantly (2–3 %), better for ICSI patients than standard IVF patients, contrary to the CDC data [40]. However, it is important to note that pregnancy rates are generally much lower than the rates reported by the CDC clinics, and this may reflect practice difference that confound the data. Nevertheless, the HFEA report states, "The difference in outcomes between ICSI and IVF most likely relates to the cause of the underlying fertility problem rather than a difference in the effectiveness of the treatment. ICSI is a more invasive treatment than IVF and should only be carried out when there is a clear medical reason to do so" [40].

Perhaps the most convincing data that ICSI is not advantageous to all patients are derived from a well-designed and powered study by Nangia et al. in which 465,046 cycles reported to the Society of Assisted Reproductive Technology (SART) clinic outcomes reporting database between 2004 and 2008 were analyzed [41]. When comparing cycles with or without ICSI only in couples with no male factor infertility and only with female tubal factor infertility, the pregnancy rate is significantly higher for non-ICSI than those undergoing ICSI (45.4 % vs. 40.4 %). Interestingly, the fetal loss/stillbirth rate is also significantly lower in the non-ICSI group (16.0 % vs. 19.3 %). This study is the largest study reported (more than 7,000 couples in the subset described above) and well defined to answer the question of if routine ICSI is generally more beneficial than standard insemination. The data imply it is not.

It is important to note that no prospective study has demonstrated a benefit for ICSI in avoiding fertilization failure in cases with low oocyte yield. In a retrospective study by Luna et al. comparing ICSI with standard insemination in cycles with four or fewer oocytes, no difference was reported in the incidence of fertilization failure, cancellation rates, or clinical pregnancy [42]. This study has recently been confirmed by Xi et al. [43]. Lastly, some have proposed that it is financially and emotionally advantageous for the couple to perform ICSI, even when no clinical indications for ICSI are present. The primary explanation for this is the possibility of fertilization failure if standard insemination is used. However, the data described above clearly show that the fertilization failure rate is not higher for standard insemination, even when the number of oocytes is diminished [42].

17.5 Conclusions

As described above, ICSI utilization is increasing and there are those who are proponents of universal ICSI for IVF patients. This trend is concurrent with another trend of minimizing the medical evaluation of male infertility patients. As stated in a report of the Bertarelli Foundation's Second Global Conference, "…the current treatment of male infertility has become so dominated by the breakthrough technology of ICSI that a kind of 'nihilism' has become widespread in the field. This cynical viewpoint could be summed up in the following words, 'As long as a few sperm are present, no further review of the male is needed'" [44]. The report then summarizes the reasons for further evaluation of the male, including his gametes, and concludes that obvious reasons include questions of health for the male and identifying relevant information regarding the treatment

options and outcomes for the couple. As stated by the HFEA, ICSI should "only be carried out when there is a clear reason to do so" [40]. The data presented above highlight the fact that indiscriminate use of ICSI is not warranted and may be disadvantageous to the patient and offspring.

Numerous studies have demonstrated the benefits of sperm function testing in assessing the need for ICSI and improving the care of patients [45]. While sperm function tests are notoriously difficult to implement and require strict quality control measures and proficiency testing, they can be useful in minimizing the risk of fertilization failure to a point that it is very cost-effective in selecting which patients actually need ICSI and fertilization failure is a rare event and usually due to oocyte immaturity rather than sperm dysfunction. There is a strong need for greater consideration more careful evaluation of the male in couples undergoing IVF, including the use of sperm function assays, strictly implemented and controlled. This change, in some clinics, may be described as a return to a more traditional medical evaluation and care. Studies and guidelines are needed in each clinic to evaluate the ramifications of nonindicated ICSI and establish useful guidelines in selecting patients for ICSI. Given the changing medical climate in the USA and some other countries, the necessity and value of measures of cost-effectiveness will likely become more emphasized in our field. Regardless of political pressure or economic realities, each clinic should perform a careful, honest evaluation of the cost/benefit ramifications of unwarranted ICSI to the patients, not just the clinics. It is doubtful that such an analysis would warrant universal ICSI or the increasing trend of increased ICSI utilization reported by the CDC [12].

In summary, ICSI is one of the most important advancements in the treatment of male factor infertility and has benefited countless couples. However, it is clear that real differences exist in the fertilization process resulting from ICSI and that gene transcription is altered in such embryos. Given our emerging understanding of the possible effects of subtle epigenetic differences on cell function and disease, further studies and long-term monitoring of ICSI offspring are warranted. Lastly, given the data we have and an understanding of the limitations of the available data, ICSI should be utilized discriminately in an evidence-based manner.

References

1. Hiramoto Y. Microinjection of the live spermatozoa into sea urchin eggs. Exp Cell Res. 1962;27:416–26.
2. Iritani A, Utsumi K, Miyake M, et al. In vitro fertilization by a routine method and by micromanipulation. Ann N Y Acad Sci. 1988;541:583–90.
3. Keefer CL. Fertilization by sperm injection in the rabbit. Gamete Res. 1989;22(1):59–69.
4. Lanzendorf S, Maloney M, Ackerman S, et al. Fertilizing potential of acrosome-defective sperm following microsurgical injection into eggs. Gamete Res. 1988;19(4):329–37.
5. Lanzendorf SE, Maloney MK, Veeck LL, et al. A preclinical evaluation of pronuclear formation by microinjection of human spermatozoa into human oocytes. Fertil Steril. 1988;49(5):835–42.
6. Fishel S, Timson J, Lisi F, et al. Micro-assisted fertilization in patients who have failed subzonal insemination. Hum Reprod. 1994;9(3):501–5.
7. Cohen J, Talansky B, Alikani M. Laboratory techniques for handling gametes and embryos. Br Med Bull. 1990;46(3):643–53.
8. Palermo G, Joris H, Devroey P, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1992;340(8810):17–8.
9. Palermo GD, Neri QV, Monahan D, et al. Development and current applications of assisted fertilization. Fertil Steril. 2012;97(2):248–59.
10. Palermo GD, Cohen J, Rosenwaks Z. Intracytoplasmic sperm injection: a powerful tool to overcome fertilization failure. Fertil Steril. 1996;65(5):899–908.
11. ESHRE. The word's number of IVF and ICSI babies has now reached a calculated total of 5 million. 2012 [2/4/2013]; Available from: http://www.eshre.eu/ESHRE/English/Press-Room/Press-Releases/Press-releases-2012/5-million-babies/page.aspx/1606.
12. CDC, ASRM, SART. 2008 Assisted reproductive technology success rates: National Summary and Fertility Clinic Reports. Atlanta: US Department of Health and Human Services 2010
13. Hansen M, Kurinczuk JJ, Bower C, et al. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. N Engl J Med. 2002;346(10):725–30.
14. Hansen M, Bower C, Milne E, et al. Assisted reproductive technologies and the risk of birth defects–a systematic review. Hum Reprod. 2005;20(2):328–38.
15. Farhi J, Fisch B. Risk of major congenital malformations associated with infertility and its treatment by

extent of iatrogenic intervention. Pediatr Endocrinol Rev. 2007;4(4):352–7.
16. Tararbit K, Lelong N, Thieulin AC, et al. The risk for four specific congenital heart defects associated with assisted reproductive techniques: a population-based evaluation. Hum Reprod. 2013;28(2):367–74.
17. Wen J, Jiang J, Ding C, et al. Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. Fertil Steril. 2012;97(6):1331–7. e1–4.
18. Stahl PJ, Schlegel PN. Genetic evaluation of the azoospermic or severely oligozoospermic male. Curr Opin Obstet Gynecol. 2012;24(4):221–8.
19. Carrell DT. The clinical implementation of sperm chromosome aneuploidy testing: pitfalls and promises. J Androl. 2008;29(2):124–33.
20. Tamburrino L, Marchiani S, Montoya M, et al. Mechanisms and clinical correlates of sperm DNA damage. Asian J Androl. 2012;14(1):24–31.
21. Barratt CL, De Jonge CJ. Clinical relevance of sperm DNA assessment: an update. Fertil Steril. 2010; 94(6):1958–9.
22. Aston KI, Carrell DT. Genome-wide study of single-nucleotide polymorphisms associated with azoospermia and severe oligozoospermia. J Androl. 2009;30(6):711–25.
23. Aston KI, Punj V, Liu L, et al. Genome-wide sperm deoxyribonucleic acid methylation is altered in some men with abnormal chromatin packaging or poor in vitro fertilization embryogenesis. Fertil Steril. 2012;97(2):285–92.
24. Aston KI, Carrell DT. Emerging evidence for the role of genomic instability in male factor infertility. Syst Biol Reprod Med. 2012;58(2):71–80.
25. Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. N Engl J Med. 2012;366(19):1803–13.
26. El Hajj N, Haaf T. Epigenetic disturbances in in vitro cultured gametes and embryos: implications for human assisted reproduction. Fertil Steril. 2013;99:632–41.
27. Jenkins TG, Carrell DT. The sperm epigenome and potential implications for the developing embryo. Reproduction. 2012;143(6):727–34.
28. Alegria-Torres JA, Baccarelli A, Bollati V. Epigenetics and lifestyle. Epigenomics. 2011;3(3):267–77.
29. Hewitson LC, Simerly CR, Tengowski MW, et al. Microtubule and chromatin configurations during rhesus intracytoplasmic sperm injection: successes and failures. Biol Reprod. 1996;55(2):271–80.
30. Sutovsky P, Hewitson L, Simerly CR, et al. Intracytoplasmic sperm injection for Rhesus monkey fertilization results in unusual chromatin, cytoskeletal, and membrane events, but eventually leads to pronuclear development and sperm aster assembly. Hum Reprod. 1996;11(8):1703–12.
31. Hewitson L, Simerly CR, Schatten G. Fate of sperm components during assisted reproduction: implications for infertility. Hum Fertil (Camb). 2002;5(3): 110–6.
32. Ajduk A, Yamauchi Y, Ward MA. Sperm chromatin remodeling after intracytoplasmic sperm injection differs from that of in vitro fertilization. Biol Reprod. 2006;75(3):442–51.
33. Finch KA, Fonseka KG, Abogrein A, et al. Nuclear organization in human sperm: preliminary evidence for altered sex chromosome centromere position in infertile males. Hum Reprod. 2008;23(6): 1263–70.
34. Hammoud SS, Nix DA, Zhang H, et al. Distinctive chromatin in human sperm packages genes for embryo development. Nature. 2009;460(7254): 473–8.
35. Jenkins TG, Carrell DT. Dynamic alterations in the paternal epigenetic landscape following fertilization. Front Genet. 2012;3:143.
36. Giritharan G, Li MW, De Sebastiano F, et al. Effect of ICSI on gene expression and development of mouse preimplantation embryos. Hum Reprod. 2010;25(12): 3012–24.
37. Bridges PJ, Jeoung M, Kim H, et al. Methodology matters: IVF versus ICSI and embryonic gene expression. Reprod Biomed Online. 2011;23(2): 234–44.
38. Kohda T, Ogonuki N, Inoue K, et al. Intracytoplasmic sperm injection induces transcriptome perturbation without any transgenerational effect. Biochem Biophys Res Commun. 2011;410(2):282–8.
39. Hill MJ, Richter KS, Heitmann RJ, et al. Trophectoderm grade predicts outcomes of singleblastocyst transfers. Fertil Steril. 2013 Jan 8. doi:pii: S0015-0282(12) 02494-6. 10.1016/j.fertnstert.2012.12.003. [Epub ahead of print] PMID: 23312233 [PubMed - as supplied by publisher]
40. HFEA. Long term trends in fertility treatment: patients treated. London: Human Fertilisation and Embryology Authority; 2011 [cited 2012 2/4/2013]; Available from: http://www.hfea.gov.uk/2585.html.
41. Nangia AK, Luke B, Smith JF, et al. National study of factors influencing assisted reproductive technology outcomes with male factor infertility. Fertil Steril. 2011;96(3):609–14.
42. Luna M, Bigelow C, Duke M, et al. Should ICSI be recommended routinely in patients with four or fewer oocytes retrieved? J Assist Reprod Genet. 2011; 28(10):911–5.
43. Xi QS, Zhu LX, Hu J, et al. Should few retrieved oocytes be as an indication for intracytoplasmic sperm injection? J Zhejiang Univ Sci B. 2012;13(9): 717–22.
44. Lunenfeld B, Van Steirteghem A. Infertility in the third millennium: implications for the individual, family and society: condensed meeting report from the Bertarelli Foundation's second global conference. Hum Reprod Update. 2004;10(4):317–26.
45. Lamb DJ. Semen analysis in 21st century medicine: the need for sperm function testing. Asian J Androl. 2010;12(1):64–70.

The Need for Long-Term Follow-Up of Children Conceived Through ICSI

18

Rachel Weinerman, Kurt T. Barnhart, and Suleena Kansal Kalra

18.1 Introduction

Intracytoplasmic sperm injection (ICSI) has become an important therapeutic intervention in assisted reproduction technologies (ART), overcoming many of the problems presented by male factor infertility. The procedure, involving the injection of a single spermatozoon into a mature oocyte, has allowed for fertilization and pregnancy even in cases of severe oligospermia or azoospermia [1]. Since its introduction in 1992, ICSI use has grown substantially and is now responsible for the majority of inseminations in many ART centers [2]; in 2010, ICSI was used in approximately 66 % of in vitro fertilization (IVF) procedures in the USA, although the diagnosis of male factor infertility was given in only 35 % of cases [3]. Although the overwhelming majority of children born following ICSI have been healthy, concerns have been raised about the potential harmful effects of ICSI on the resulting offspring, and these have been borne out by multiple studies in several specific areas [4]. This chapter reviews the literature to date on the outcomes of children born following ICSI, with emphasis on the incidence of congenital abnormalities, imprinting deficiencies, chromosomal and karyotypic abnormalities, and neurologic and developmental outcomes. Continued follow-up of these children is essential to determine the long-term outcomes of children conceived through ICSI.

18.2 Congenital Anomalies

A major controversy surrounding IVF and ICSI since its inception has been the outcome of children born following ART procedures [5]. Although the absolute risk still remains small, most studies have shown an increase in congenital malformations following ART [6–9]. A recent meta-analysis analyzing birth defects following IVF, ICSI, and natural cycle showed a relative risk of birth defects following ART of 1.39 (85 % CI 1.29–1.50) [10].

Whether there is an additional increased risk of congenital malformations following ICSI is more controversial (Table 18.1). A systematic

R. Weinerman, M.D. • S.K. Kalra, M.D., M.S.C.E.
Penn Fertility Care, Department of Obstetrics and Gynecology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

K.T. Barnhart, M.D., M.S.C.E. (✉)
Penn Fertility Care, Department of Obstetrics and Gynecology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Women's Health Clinical Research Center, Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA
e-mail: kbarnhart@obgyn.upenn.edu

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_18,

Table 18.1 Selected studies of congenital malformations in ICSI offspring

Citation	Methods	Outcome: OR (95 % CI)	Findings
Sutcliffe et al. 2001 [14]	Cohort study of 208 ICSI singletons and 221 controls	1.76 (0.87–3.54)	Higher rates of malformations seen in children born from men with oligospermia
Rimm et al. 2004 [6]	Meta-analysis of 16 studies of children from IVF (n=28,524) versus controls (n=2,520,988) and ICSI (n=7,234) versus controls (n=978,078)	Overall: 1.29 (1.01–1.67)	ICSI malformation rates 1–1–9.7 %; no differences seen in malformation rates between IVF and ICSI
Hansen et al. 2005 [7]	Systematic review of 25 published studies through March 2003; meta-analysis of seven studies	IVF/ICSI: 2.01 (1.49–2.69) ICSI only: 2.0 (1.3–3.2) 1.28 (1.14–1.43)	Overall studies suggest increased rate of malformations of 30–40 % following ART; similar rates of malformations seen in IVF versus ICSI
Fedder et al. 2007 [16]	Retrospective questionnaire study of 412 ICSI children	N/A	1.6 % rate of hypospadias, increased relative to the general population
Palermo et al. 2008 [5]	Retrospective review of 5,891 ICSI and 3,893 IVF children	No differences	No differences in rates of major and minor anomalies between IVF and ICSI children
Kallen et al. 2010 [12]	Retrospective review of 11,642 ICSI and 20,483 IVF births in large Swedish registry	Overall: 1.15 (1.07–1.24)	No differences in malformation rates between IVF and ICSI; no differences seen between ejaculated and nonejaculated sperm
Woldringh et al. 2010 [11]	Meta-analysis of eight studies of children from ICSI with nonejaculated sperm (n=1,123) versus ICSI with ejaculated sperm (n=12,377)	No differences between the groups	No differences in malformations rates between IVF and ICSI and between ejaculated ICSI and nonejaculated ICSI
Belva et al. 2011 [18]	Retrospective study of ICSI children; 2,516 ejaculated sperm and 724 nonejaculated sperm	Ejaculated versus nonejaculated sperm: 1.4 (0.9–2.2)	Increased rate of genital tract anomalies in nonejaculated sperm group (OR 3.0, CI 1.0–9.2)
Davies et al. 2012 [13]	Australian birth registry data; 1,484 IVF and 939 ICSI births; 293,314 fertile controls, 1,906 subfertile controls	ICSI singletons: 1.55 (1.24–1.94)	Increased risk of musculoskeletal abnormalities (OR 1.5), urogenital abnormalities (OR 1.25), and cardiovascular abnormalities (OR 1.36)
Fedder et al. 2012 [17]	Cohort study of Danish children; 466 ICSI nonejaculated sperm; 8,967 ICSI ejaculated sperm; 17,592 IVF, 63,854 controls	No OR given	Increased risk of cardiac malformations in nonejaculated ICSI compared to IVF, increased trend of undescended testicles across the groups
Wen et al. 2012 [10]	Meta-analysis of 46 studies comparing IVF/ICSI (n=124,468) to spontaneously conceived children, 24 studies comparing IVF children (n=46,890) to ICSI children (n=27,754)	IVF/ICSI to control: 1.37 (1.26–1.48) IVF to ICSI: No difference	Increased risk of nervous system, genitourinary, and other defects between IVF/ICSI and control, no increased risk between IVF and ICSI

review of five studies evaluating congenital anomalies in children born after ICSI with ejaculated and nonejaculated sperm showed no differences in anomaly rates based on sperm origin, although each study was small and the studies overall were heterogeneous; additionally, three of the five studies compared outcomes of ICSI versus IVF and showed no differences in malformation rates [11]. A large study of 5,891 babies resulting from ICSI and 3,893 babies resulting from IVF found no difference in congenital anomalies between the two groups, with rates of 3.5 % and 3.4 %, respectively [5]. Another large study of 31,850 infants born in Sweden after IVF or ICSI showed no differences in the rates of rates of anomalies between the two groups [12].

In contrast, a recent large study of birth registries in Australia found an increased risk of birth defects after IVF that was not significant after controlling for parental factors; however, the increased risk of birth defects following ICSI remained significant, with an adjusted odds ratio (OR) of 1.57 (95 % CI 1.3–1.9). The most commonly reported malformations in the above-noted study included musculoskeletal abnormalities (OR 1.5), urogenital abnormalities (OR 1.25), and cardiovascular abnormalities (OR 1.36) [13]. A study of 208 singleton children following ICSI found an increased rate of congenital anomalies among children born to oligospermic fathers compared to children conceived with ICSI whose fathers did not have oligospermia [14].

Hypospadias and male urogenital anomalies have been suggested to be associated with ICSI, due to the prevalence of defects in spermatogenesis and male infertility in the fathers of ICSI offspring [15]. A survey study of 412 children born following ICSI with epididymal or testicular sperm (retrieved using testicular sperm extraction (TESE) or percutaneous epididymal sperm aspiration (PESA)) found hypospadias in 3 out of 187 boys for a rate of 1.6 %, five times the expected rate of 0.28 % in the general population [16]. A recent cohort study compared 486 children born following ICSI with sperm retrieved surgically [TESE, PESA, or testicular sperm aspiration (TESA)] to 8,967 children from ICSI with ejaculated sperm, 15,592 children from IVF, and 63,854 natural cycle controls. The study showed overall similar rate of congenital malformations between the groups; however, the rate of cardiac malformations was higher in the surgical retrieval group compared to the IVF and (3.6 % vs. 1.1–1.4 %), and an increase in the rate of undescended testicles was seen progressively across the four groups. The hypospadias rate did not differ significantly in the surgical retrieval group compared to the control groups [17]. An analysis of 724 consecutive births following ICSI using ejaculated and nonejaculated sperm showed a higher rate of major genital tract anomalies, including cryptorchid testes and hypospadias, in the nonejaculated versus ejaculated groups (1 % vs. 0.3 %, OR 3.0 CI 1.0–9.2) [18].

Although the vast majority of children conceived via ICSI do not have congenital anomalies, further studies are needed to ascertain the risk of birth defects and the possible etiologies for the differences seen in large, population-based studies.

18.3 Imprinting

Imprinting is a process of epigenetic changes to a genome, whereby genes are modified according to the parental origin of each allele, often by DNA methylation or histone modifications [19]. A small but significant increase in imprinting disorders has been associated with ART procedures and ICSI, including Beckwith–Wiedemann Syndrome, Angelman Syndrome, and retinoblastoma (Table 18.2).

There are approximately 40 imprinted genes in humans [20]. In these genes, one allele, determined by its parental origin, is silenced. Imprinting marks are erased in germ cells but are reestablished during gametogenesis. Complete establishment of imprinting in the oocyte does not occur until oocyte maturation; the process occurs earlier in sperm development [21].

There are multiple ways in which ART may affect genomic imprinting, including superovulation, in vitro culture of embryos, and in vitro maturation of oocytes. Studies involving mouse models have, in fact, shown associations between

Table 18.2 Imprinting disorders and IVF/ICSI

Disorder	Gene affected	Number of cases
Beckwith–Wiedemann Syndrome	*DMR1*, *DMR2* (maternal methylation)	64/656 cases following ART (30 ICSI, 34 IVF) [27]
Angelman Syndrome	*UBE3A* (hypomethylation of maternal allele)	Seven cases following ART (6 ICSI, 1 IVF) [19]
Retinoblastoma	*RB1* (proposed mechanism hypermethylation of promoter gene; not supported by gene sequencing studies) [35]	Nine reported by Moll and colleagues (3 ICSI, 6 IVF) [31, 34, 35]

disruptions of methylated genes, including H19, IGF2, and CDKN1C and all of the above interventions [5, 19]. ICSI specifically may also affect imprinting because of its reliance on sperm from men with oligospermia. Multiple studies have shown an association between oligospermia and male infertility and abnormal gene methylation patterns including methylation changes in the genes H19, MEST, HUMARA, and SNPRN [22–25]. Clinically, there have also been several case series suggesting an association between ICSI and rare imprinting disorders, as discussed below.

Beckwith–Wiedemann Syndrome (BWS), a rare developmental and growth disorder with an incidence of approximately 1:13,700 in the general population, has been associated with ART, with a relative risk estimated to be six to nine times higher among ART offspring [4]. Multiple case series have suggested an association between ART and BWS; initially, these series suggested an association with ICSI specifically, but more recent reports have shown an association between BWS and IVF or ICSI [19, 26]. These series of BWS cases showed a higher incidence of IVF or ICSI use in the cases than would be expected in the general population based on ART rates at the time; a total of 64 out of 656 cases of BWS followed ART, for a rate of 9.7 % compared to ~0.007 % in the general population [27]. Importantly, 90–100 % of the cases of BWS after ART tested in these series had an imprinting defect (mainly methylation of the maternal allele) compared to the expected rate of 50 % in the general population [19]. Of these 64 cases, 30 (47 %) followed ICSI and 34 (53 %) followed IVF [27]. Additionally, a recent study of BWS children that compared 25 post-ART children (12 IVF, 13 ICSI) to non-ART BWS cases showed more methylation deficiencies in the ART cases, again suggesting an association between IVF/ICSI and defects in imprinting [28].

Angelman Syndrome (AS), another imprinting disorder characterized by severe mental retardation, seizures, and motor difficulties, has a more specific association with ICSI. AS, with an incidence of approximately 1 in 12,000 in the general population, is due to loss of maternal gene expression of the UBE3A gene [29]. An imprinting defect in this gene is generally responsible for only 5 % of cases of AS [30]; however, five of the seven cases of AS that have been reported following IVF or ICSI have had an imprinting defect, for a rate of 71 %; six out of these cases have followed ICSI (86 %) and one followed IVF (14 %) [19].

Retinoblastoma is another condition thought to be associated with imprinting that has been linked to ART. A malignant tumor of the retina, retinoblastoma has an incidence of approximately 1:17,000 births [31]. Retinoblastoma is most often caused by a mutation in one allele of the tumor suppressor gene *RB1*; loss of the second allele is followed by tumor formation [32]. However, in 10–12 % of retinoblastoma cases, the mutation in the *RB1* gene is due to hypermethylation of the promoter gene [33], implying an association with imprinting. A study in the Netherlands in 2003 reported five cases of retinoblastoma following IVF in 3 years. The authors calculated a relative risk of five to seven based on the prevalence of IVF of 1–1.5 % at that time [31]. In this series, four of the cases followed IVF and one followed ICSI. In a follow-up study with data from 1995 to 2007, seven cases of retinoblastoma were identified following IVF (five IVF and two ICSI), for a relative risk (RR) of 2.5 during the entire study period [34]. Since then, two

more cases have been identified following ART: one from IVF and one from ICSI; for a total of nine cases: six following IVF and three following ICSI [35]. However, in their follow-up study, the authors performed gene sequencing on the tumors of these patients and did not find hypermethylation of the promoter gene to be a cause of the mutation in any of the cases of retinoblastoma following IVF/ICSI, thus proving that the association between IVF/ICSI and retinoblastoma is not related to epigenetic changes; the etiology of this association therefore remains unknown [35].

Although the association between imprinting and ART is stronger than that for imprinting and ICSI specifically, the theoretical concern exists that ICSI may have an increased risk of imprinting disorders due to oligospermia and its association with impaired methylation [25]. Additionally, multiple studies have shown associations between imprinting disorders and a history of infertility in the parents, regardless of ultimate conception method [36–38]. Population studies have been limited by the rare nature of these disorders, with reports from multiple large registries failing to show an increase in the relative risk of imprinting disorders [39, 40]. However, with the incidence of BWS, AS, and retinoblastoma each in range of 1:12,000–1:17,000, it would be difficult to show a significant difference without an extremely large patient population [34].

Imprinting changes related to ART may have subtler clinical effects as well, including changes in growth, metabolism, and neurocognitive development [26]. Additionally, there have been some studies suggesting an increase in child malignancies following ART [41, 42], a finding that may be related to imprinting changes in tumor suppressor genes [43].

The association of imprinting with ART is still unclear. Initial studies suggested that ICSI contributed to increased risk, but more recent studies have refuted that. The risk of imprinting disorders following ART is an area of continued, active investigation and long-term follow-up is needed to better ascertain the actual incidence as well as the underlying etiology of risk in IVF- and ICSI-conceived children

18.4 Chromosomal Abnormalities

Special attention has been paid to the karyotypes and chromosomal makeup of children born following ICSI due to the concern that abnormal sperm may be injected during the ICSI procedure, leading to an increased risk of chromosomal abnormalities in ICSI offspring [44]. Sperm aneuploidy has been associated with compromised sperm motility and morphology as well as sperm concentration, with aneuploidy rates highest for sperm obtained through testicular extraction [5]. Several studies have shown increased rates of chromosomal aneuploidy following ICSI, generally showing aneuploidy rates ranging from 2 to 3.5 % [45, 46] (Table 18.3).

A systematic review of the literature involving ICSI after nonejaculated sperm found two studies that specifically studied karyotypes of fetuses conceived after ICSI using ejaculated and nonejaculated sperm [11]. One study analyzed the karyotypes of 1,136 ICSI fetuses via amniocentesis. Of the patient population, 56 % had male factor infertility, 24 % female factors, and 20 % unexplained; 128 of the ICSI procedures had been performed using testicular sperm. Karyotypic abnormalities were found in 17 of the fetuses (1.5 %), seven were sex chromosomal and ten autosomal [44]. This rate is higher than the approximately 0.9 % expected in a general population [47]. There was no difference in ejaculated versus nonejaculated sperm, although the absolute number of abnormalities following nonejaculated sperm was small (3, 2.3 %). The study population was young (mean maternal age 32 years), and advanced maternal or paternal age did not correlate with the occurrence of abnormal karyotypes. When broken down by etiology of infertility, the rate of chromosomal abnormalities was 1.8 % in the male infertility group and 0.9 % in the non-male infertility group, although this difference was not statistically significant [44].

In a second study, a follow-up of a large Belgium cohort, 1,586 ICSI fetuses were tested via prenatal diagnosis; 47 (2.9 %) were abnormal, of which 25 (1.6 %) were de novo abnormalities

Table 18.3 Selected studies of chromosomal abnormalities in ICSI offspring

Citation	Methods	Findings
Bonduelle et al. 1999 [46]	Prospective study of 1,082 ICSI pregnancies with prenatal karyotype	Overall 2.6 % abnormal karyotypes; 1.66 % de novo mutations, 0.92 % inherited mutations
Aboulghar et al. 2001 [45]	Prospective study of karyotypes of 430 ICSI babies and 430 controls	Abnormal karyotype in 3.5 % of ICSI babies and none of the controls; half were sex-chromosome abnormalities (RR = 3.10, CI = 1.86–516.5)
Bonduelle et al. 2002 [48]	Prospective study of 1,586 ICSI pregnancies with prenatal karyotype	1.6 % rate of chromosomal abnormalities; higher rate noted in pregnancies conceived from fathers with sperm concentration <20 million/mL (2.1 % vs. 0.24 %)
Jozwiak et al. 2004 [44]	Prospective study of 1,136 ICSI pregnancies with prenatal karyotype	1.5 % rate of chromosomal abnormalities, no differences in rates based on etiology of infertility or origin of sperm

(ten sex chromosomal and 15 autosomal), and 22 were inherited, of which 17 were noted to be transmitted through the father. Two of the anomalies were found in fetuses of patients who had used testicular sperm. The rate of de novo abnormalities was noted to higher than that expected in the general population (1.6 % vs. 0.5 % for a population with an average age of 35.5). When analyzed by male sperm concentration, a higher rate of de novo abnormalities was seen in fetuses conceived using sperm with concentrations less than 20 million/mL compared to fetuses conceived with sperm with concentrations greater than 20 million/mL (2.1 % vs. 0.24 %, $p = 0.006$) [48].

Taken together, these studies indicate an increased risk of de novo and inherited chromosomal abnormalities following ICSI, although the absolute risk remains small. The studies also suggest that the abnormalities may be due to sperm quality and the increased likelihood of chromosomal abnormalities in men with severe male factor infertility rather than the ICSI procedure itself; however, larger sample sizes would be needed to determine this since the rate of chromosomal abnormalities is small in any given population.

18.5 Cognitive and Neurodevelopmental Deficiencies

A final area of concern regarding ICSI offspring relates to their cognitive and developmental function. While many studies have shown reassuring results of the developmental health of children born to ICSI [49–51], a few studies have shown differences in cognition following ICSI [52, 53] (Table 18.4).

An early follow-up study of 89 children born following ICSI compared to IVF and natural conception controls showed a lower mean score on the mental development index (MDI) of the Bayley Scales of Infant Development at 1 year of life; although most ICSI children fell within the normal range, a mean difference of six points lower was observed [52]. Another study of 83 ICSI singletons compared to IVF singletons showed lower IQ scores using the Revised Amsterdam Child Intelligence Test for the ICSI singletons at age 5–8 compared to the IVF controls, with a mean score 3.6 points lower when adjusted for differences between the groups; however, this difference was not statistically significant [53].

A recent study compared the motor, mental, and language development of 148 children conceived with ICSI using epididymal sperm compared Dutch reference values at 2 years of age. There were no negative differences seen in the group compared to the Dutch reference values; in fact, the MDI mean score was higher in the ICSI group, although still within the normal range ($p < 0.05$) [54]. Other smaller studies have shown no differences in cognitive development following ICSI [50, 51, 55].

Cerebral palsy (CP) has also been associated with ART; a meta-analysis of studies comparing outcomes following IVF/ICSI showed increased rates of CP following IVF or ICSI, but these differences most often disappeared after controlling

Table 18.4 Selected studies of neurodevelopment and ICSI offspring

Citation	Methods	Test used	Findings
Bowen et al. 1998 [52]	Prospective study of children conceived by ICSI ($n=89$), IVF ($n=84$), and natural conception (NC) ($n=80$) at 1 year old	Bayley Scales of Infant Development; Mental Development Index (MDI)	Mean MDI six points lower in ICSI group compared to IVF and NC ($p<0.0001$)
Knoester et al. 1998 [53]	Cohort study of singletons from ICSI ($n=83$) matched to IVF ($n=83$) and NC controls ($n=85$) at 5–8 years old	Revised Amsterdam Child Intelligence Test	Mean IQ score was 3.9 points lower in ICSI group compared to IVF (CI −0.7,8.4) and 6.8 points lower compared to controls (CI 2.0, 11.6)
Bonduelle et al. 2003 [49]	Prospective cohort study of children conceived from ICSI ($n=439$) and IVF ($n=207$) at 24–28 months	Bayley Scales of Infant Development; MDI	No differences between groups
Leslie et al. 2003 [50]	Cohort study of children conceived from ICSI ($n=97$), IVF ($n=80$), and NC ($n=110$) at 5 years old	Wechsler Preschool and Primary Scales of Intelligence	No differences in IQ scores between groups; no increased risk of delayed cognitive development in ICSI group
Sutcliffe et al. 2003 [51]	Cohort study of singletons from ICSI ($n=266$) and matched NC controls ($n=259$) at a mean age of 13 months	Griffiths Mental Development Scales	No differences between groups
Middelburg et al. 2008 [56]	Meta-analysis of 23 studies comparing neurodevelopmental outcomes in IVF/ICSI to NC controls	N/A	No association of IVF/ICSI to mental delays; association of IVF/ICSI to cerebral palsy related to other risk factors (preterm birth and multiple gestations); most studies were of infants only
Hvidtjorn et al. 2009 [57]	Meta-analysis of studies of children born from IVF/ICSI and cerebral palsy (nine studies), autism (eight studies), and developmental delay (30 studies)	N/A	Increased risk of cerebral palsy after IVF/ICSI associated with preterm delivery (OR 2.18, CI 1.71–2.77); inconsistent results in studies of autism and developmental delays and IVF/ICSI
Woldringh et al. 2011 [54]	Dutch study of 148 children conceived from ICSI using nonejaculated sperm (PESA) at 2 years old	Bayley Scales of Infant Development; Mental Development Index (MDI)	Mean MDI for PESA group higher than Dutch references ($p<0.05$)

for prematurity and multiple birth, and there was no difference seen between IVF and ICSI [56]. However, the recent large study of birth registries in Australia, which showed an increase rate of birth defects following ICSI (but not IVF), showed higher rates of CP following ART even in singleton births, with an adjusted OR of 2.22 (95 % confidence interval (CI) 1.35–3.63) [13]. Studies of autism following IVF or ICSI have been limited but a meta-analysis showed no association of autism and ART [57].

A major limitation of studies to date has been the lack of long-term follow-up. This is especially important for assessment of cognitive and neurodevelopmental delays as many do not present until school age or beyond [56]; peak prevalence for the diagnosis of autism, for example, is 8 years old [58]. Given the length of time to diagnosis of neurodevelopmental disorders, studies to assess large cohorts with appropriate controls need to be undertaken, especially as the offspring proceed until late childhood and adulthood.

18.6 Conclusion

Thousands of children have now been born following ICSI, and the large majority of these children have had favorable outcomes and normal development. Real concerns exist, however, especially with regard to the increase in congenital malformations, karyotypic abnormalities, and imprinting changes following ICSI. Imprinting defects in particular may have long-term consequences that may as yet not be known. The oldest children born from ICSI are now 20 years old; how these children will fare in their own reproductive lives and in long-term outcomes including aging and the risk of malignancy and chronic disease has yet to be determined. Care should be taken to understand the potential risks involved and counsel patients appropriately when a decision to use ICSI is made. ICSI is now performed in a majority of IVF cases, even though the efficacy of ICSI for indications other than male factor infertility remains unproven. In fact, a recent evaluation of SART data presented at ASRM demonstrated lower pregnancy rates when ICSI was performed for a variety of indications other than male factor infertility [59]. Given the potential risks and unproven efficacy, ICSI should be used judiciously and only when indicated. Long-term follow-up is essential to fully understand the implications of ICSI.

References

1. Practice Committee of the American Society for Reproductive Medicine. Does intracytoplasmic sperm injection (ICSI) carry inherent genetic risks? Fertil Steril. 2004;82 (Suppl 1):S151–2.
2. Palermo GD, Neri QV, Monahan D, et al. Development and current applications of assisted fertilization. Fertil Steril. 2012;97(2):248–59.
3. [SART National Data Summary (Internet) Cited 2010 December 5]; Available from: https://http://www.sartcorsonline.com/rptCSR_PublicMultYear.aspx?ClinicPKID=0.
4. Talaulikar VS, Arulkumaran S. Reproductive outcomes after assisted conception. Obstet Gynecol Surv. 2012;67(9):566–83.
5. Palermo GD, Neri QV, Takeuchi T, et al. Genetic and epigenetic characteristics of ICSI children. Reprod Biomed Online. 2008;17(6):820–33.
6. Rimm AA, Katayama AC, Diaz M, et al. A meta-analysis of controlled studies comparing major malformation rates in IVF and ICSI infants with naturally conceived children. J Assist Reprod Genet. 2004; 21(12):437–43.
7. Hansen M, Bower C, Milne E, et al. Assisted reproductive technologies and the risk of birth defects–a systematic review. Hum Reprod. 2005;20(2): 328–38.
8. Schieve LA, Rasmussen SA, Reefhuis J. Risk of birth defects among children conceived with assisted reproductive technology: providing an epidemiologic context to the data. Fertil Steril. 2005;84(5):1320–4. discussion 7.
9. Zhu JL, Basso O, Obel C, et al. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. BMJ. 2006;333(7570):679.
10. Wen J, Jiang J, Ding C, et al. Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. Fertil Steril. 2012;97(6):1331–7.
11. Woldringh GH, Besselink DE, Tillema AH, et al. Karyotyping, congenital anomalies and follow-up of children after intracytoplasmic sperm injection with non-ejaculated sperm: a systematic review. Hum Reprod Update. 2010;16(1):12–9.
12. Kallen B, Finnstrom O, Lindam A, et al. Congenital malformations in infants born after in vitro fertilization in Sweden. Birth Defects Res A Clin Mol Teratol. 2010;88(3):137–43.

13. Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. N Engl J Med. 2012;366(19):1803–13.
14. Sutcliffe AG, Taylor B, Saunders K, et al. Outcome in the second year of life after in-vitro fertilisation by intracytoplasmic sperm injection: a UK case–control study. Lancet. 2001;357(9274):2080–4.
15. Halliday J. Outcomes for offspring of men having ICSI for male factor infertility. Asian J Androl. 2012;14(1):116–20.
16. Fedder J, Gabrielsen A, Humaidan P, et al. Malformation rate and sex ratio in 412 children conceived with epididymal or testicular sperm. Hum Reprod. 2007;22(4):1080–5.
17. Fedder J, Loft A, Parner ET, et al. Neonatal outcome and congenital malformations in children born after ICSI with testicular or epididymal sperm: a controlled national cohort study. Hum Reprod. 2012;28: 230–40.
18. Belva F, De Schrijver F, Tournaye H, et al. Neonatal outcome of 724 children born after ICSI using non-ejaculated sperm. Hum Reprod. 2011;26(7):1752–8.
19. Manipalviratn S, DeCherney A, Segars J. Imprinting disorders and assisted reproductive technology. Fertil Steril. 2009;91(2):305–15.
20. Morison IM, Ramsay JP, Spencer HG. A census of mammalian imprinting. Trends in Genetics: TIG. 2005;21(8):457–65.
21. Hartmann S, Bergmann M, Bohle RM, et al. Genetic imprinting during impaired spermatogenesis. Mol Hum Reprod. 2006;12(6):407–11.
22. Marques CJ, Carvalho F, Sousa M, et al. Genomic imprinting in disruptive spermatogenesis. Lancet. 2004;363(9422):1700–2.
23. Kobayashi H, Sato A, Otsu E, et al. Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients. Hum Mol Genet. 2007;16(21): 2542–51.
24. Dasoula A, Georgiou I, Kontogianni E, et al. Methylation status of the SNRPN and HUMARA genes in testicular biopsy samples. Fertil Steril. 2007;87(4):805–9.
25. Marques CJ, Costa P, Vaz B, et al. Abnormal methylation of imprinted genes in human sperm is associated with oligozoospermia. Mol Hum Reprod. 2008; 14(2):67–74.
26. Maher ER. Imprinting and assisted reproductive technology. Hum Mol Genet. 2005;14(Spec No 1):133–8.
27. Eroglu A, Layman LC. Role of ART in imprinting disorders. Semin Reprod Med. 2012;30(2):92–104.
28. Lim D, Bowdin SC, Tee L, et al. Clinical and molecular genetic features of Beckwith–Wiedemann syndrome associated with assisted reproductive technologies. Hum Reprod. 2009;24(3):741–7.
29. Mann MR, Bartolomei MS. Towards a molecular understanding of Prader-Willi and Angelman syndromes. Hum Mol Genet. 1999;8(10):1867–73.
30. Cox GF, Burger J, Lip V, et al. Intracytoplasmic sperm injection may increase the risk of imprinting defects. Am J Hum Genet. 2002;71(1):162–4.
31. Moll AC, Imhof SM, Cruysberg JR, et al. Incidence of retinoblastoma in children born after in-vitro fertilisation. Lancet. 2003;361(9354):309–10.
32. Thompson JR, Williams CJ. Genomic imprinting and assisted reproductive technology: connections and potential risks. Semin Reprod Med. 2005;23(3): 285–95.
33. Zeschnigk M, Bohringer S, Price EA, et al. A novel real-time PCR assay for quantitative analysis of methylated alleles (QAMA): analysis of the retinoblastoma locus. Nucleic Acids Res. 2004;32(16):e125.
34. Marees T, Dommering CJ, Imhof SM, et al. Incidence of retinoblastoma in Dutch children conceived by IVF: an expanded study. Hum Reprod. 2009;24(12): 3220–4.
35. Dommering CJ, van der Hout AH, Meijers-Heijboer H, et al. IVF and retinoblastoma revisited. Fertil Steril. 2012;97(1):79–81.
36. Draper ES, Kurinczuk JJ, Abrams KR, et al. Assessment of separate contributions to perinatal mortality of infertility history and treatment: a case–control analysis. Lancet. 1999;353(9166):1746–9.
37. Neelanjana M, Sabaratnam A. Malignant conditions in children born after assisted reproductive technology. Obstet Gynecol Surv. 2008;63(10):669–76.
38. Doornbos ME, Maas SM, McDonnell J, et al. Infertility, assisted reproduction technologies and imprinting disturbances: a Dutch study. Hum Reprod. 2007;22(9):2476–80.
39. Lidegaard O, Pinborg A, Andersen AN. Imprinting diseases and IVF: Danish National IVF cohort study. Hum Reprod. 2005;20(4):950–4.
40. Kallen B, Finnstrom O, Nygren KG, et al. In vitro fertilization (IVF) in Sweden: risk for congenital malformations after different IVF methods. Birth Defects Res A Clin Mol Teratol. 2005;73(3):162–9.
41. Klip H, Burger CW, de Kraker J, et al. Risk of cancer in the offspring of women who underwent ovarian stimulation for IVF. Hum Reprod. 2001;16(11): 2451–8.
42. Bruinsma F, Venn A, Lancaster P, et al. Incidence of cancer in children born after in-vitro fertilization. Hum Reprod. 2000;15(3):604–7.
43. Lim DH, Maher ER. Genomic imprinting syndromes and cancer. Adv Genet. 2010;70:145–75.
44. Jozwiak EA, Ulug U, Mesut A, et al. Prenatal karyotypes of fetuses conceived by intracytoplasmic sperm injection. Fertil Steril. 2004;82(3):628–33.
45. Aboulghar H, Aboulghar M, Mansour R, et al. A prospective controlled study of karyotyping for 430 consecutive babies conceived through intracytoplasmic sperm injection. Fertil Steril. 2001;76(2):249–53.
46. Bonduelle M, Camus M, De Vos A, et al. Seven years of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. Hum Reprod. 1999;14 Suppl 1:243–64.
47. Jacobs PA, Browne C, Gregson N, et al. Estimates of the frequency of chromosome abnormalities detectable in unselected newborns using moderate levels of banding. J Med Genet. 1992;29(2):103–8.

48. Bonduelle M, Liebaers I, Deketelaere V, et al. Neonatal data on a cohort of 2889 infants born after ICSI (1991–1999) and of 2995 infants born after IVF (1983–1999). Hum Reprod. 2002;17(3):671–94.
49. Bonduelle M, Ponjaert I, Steirteghem AV, et al. Developmental outcome at 2 years of age for children born after ICSI compared with children born after IVF. Hum Reprod. 2003;18(2):342–50.
50. Leslie GI, Gibson FL, McMahon C, et al. Children conceived using ICSI do not have an increased risk of delayed mental development at 5 years of age. Hum Reprod. 2003;18(10):2067–72.
51. Sutcliffe AG, Saunders K, McLachlan R, et al. A retrospective case–control study of developmental and other outcomes in a cohort of Australian children conceived by intracytoplasmic sperm injection compared with a similar group in the United Kingdom. Fertil Steril. 2003;79(3):512–6.
52. Bowen JR, Gibson FL, Leslie GI, et al. Medical and developmental outcome at 1 year for children conceived by intracytoplasmic sperm injection. Lancet. 1998;351(9115):1529–34.
53. Knoester M, Helmerhorst FM, Vandenbroucke JP, et al. Cognitive development of singletons born after intracytoplasmic sperm injection compared with in vitro fertilization and natural conception. Fertil Steril. 2008;90(2):289–96.
54. Woldringh GH, Horvers M, Janssen AJ, et al. Follow-up of children born after ICSI with epididymal spermatozoa. Hum Reprod. 2011;26(7):1759–67.
55. Sanchez-Albisua I, Lidzba K, Borell-Kost S, et al. Medical, psychological and intellectual development of 5-year-old children born after intracytoplasmic sperm injection. Neuropediatrics. 2011;42(3):104–9.
56. Middelburg KJ, Heineman MJ, Bos AF, et al. Neuromotor, cognitive, language and behavioural outcome in children born following IVF or ICSI-a systematic review. Hum Reprod Update. 2008;14(3):219–31.
57. Hvidtjorn D, Schieve L, Schendel D, et al. Cerebral palsy, autism spectrum disorders, and developmental delay in children born after assisted conception: a systematic review and meta-analysis. Arch Pediatr Adolesc Med. 2009;163(1):72–83.
58. Autism, Developmental Disabilities Monitoring Network Surveillance Year Principal I, Centers for Disease C et al. Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2008. MMWR Surveill Summ. 2008;61(3):1–19.
59. Nangia AK, Luke B, Abdel Megid W, et al. National study of factors influencing assisted reproductive technology (ART) outcomes with male factor infertility. Fertil Steril. 2010;94(4):S33.

Popularity of ICSI

19

Gianpiero D. Palermo, Queenie V. Neri, Trina Fields, and Zev Rosenwaks

19.1 Male Infertility Diagnosis

Infertility is commonly defined as the failure of conception after 12 months of unprotected intercourse [1]. Of the 62 million American women in reproductive age (15–44 years old) about 11.1 million of them have impaired fecundity. In this group, 1.2 million had infertility related consultations with an additional 6.2 million actually receiving some form of infertility treatment. It is now widely accepted that infertility causes are equally distributed between male and female factors with the remainder including combined factors or unexplained [2].

In the general population, about 25 % of couples do not achieve pregnancy within 1 year, 15 % seek treatment for infertility, and less than 5 % remain childless. According to varying surveys, it appears that the male partner is the culprit in about half of the infertility cases. Male factor infertility is commonly defined in terms of the conventional semen profile, which provides descriptive information on the numbers of spermatozoa present in the ejaculate, the proportion that are motile or progressively motile, and the percentage of morphologically normal [3]. The diagnosis of male factor infertility appears straightforward by assessing the standard semen parameters; however, the etiology remains still unclear. In fact, the underlying reasons for oligo-astheno-terato-zoospermia (OAT) are still unknown and often progressive, rendering the effectiveness of conventional treatments aimed at sperm preparation extremely doubtful. Reports have drawn attention to the genetic etiology of the loss of spermatozoal function [4], however, while the reason for primary or secondary male infertility remains a field that requires continuous understanding. The implementation of micromanipulation techniques in the last 20 years has made it possible to overcome male gamete production and fertilization defects to allow male partners to reproduce at rates that previously would have been deemed unachievable.

The position of andrology in the male infertility work-up has been strengthened by the introduction of testicular sperm extraction (TESE) and microsurgical epididymal sperm aspiration (MESA) following appropriate differential diagnosis of infertility causes (obstructive/nonobstructive azoospermia, congenital bilateral absence of the vas deferens) or acquired, respectively. The effectiveness of treating varicoceles in terms of pregnancy outcome is still a matter of

G.D. Palermo, Ph.D., M.D. (✉) • Q.V. Neri, M.Sc.
T. Fields, B.S. • Z. Rosenwaks, M.D.
The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, 1305 York Avenue, Suite 720, New York, NY 10021, USA
e-mail: gdpalerm@med.cornell.edu

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_19,

discussion. However, it seems clear that spermatogenesis can be, at least partially, restored, after microsurgical varicocelectomy in some men with cryptozoospermia or secretory azoospermia [5].

While obtaining an infertility history, it is important to attempt to identify any risk factors (e.g., cryptorchidism and environmental hazards) that may be of aid in their andrological assessment. Unfortunately, the overzealous enthusiasm of the reproductive specialists may drive the excessive use of unconfirmed diagnostic tests. In fact, these tests that claim to predict fertility status or to indicate the appropriate ART procedure risk to emotionally and financially drain childless men.

More relevant would be the routine genetic analysis for clinical diagnosis of nonobstructive azoospermia or severe oligozoospermia such as the assessment for presence of microdeletions on the long arm of the Y chromosome (Yq). The first genetic test should be a karyotype analysis where about 5 % of patients with fertility issues would be identified and the prevalence increases to around 15 % when considering men with azoospermia [6]. Besides numerical abnormalities, structural defects are also detected 5–10 times more frequently in infertile men [7]. In addition, other genes associated with normal sexual development, testis determination, testis descent, and spermatogenesis should be assessed such as the CFTR (cystic fibrosis transmembrane conductance regulator) gene whose mutation cause cystic fibrosis and absence of the vas deferens; the androgen receptor gene whose mutations may cause androgen insensitivity syndrome along with spermatogenic damage; and the INSL3 (insulin-like factor 3) and LGR8 (leucine-rich repeat-containing G-protein coupled receptor 8) genes whose mutations are associated with abnormalities in testis descent [7]. In some rare cases, men with a well-defined sperm abnormality such as globozoospermia can be assessed for single gene defects, such as SPATA16 (spermatogenesis associated protein 16) [8] and PICK1 (protein interacting with c kinase 1) [9], which are both presumably involved in the formation of the acrosome. Single gene defects are commonly expected in patients with a specific phenotype. However, the large majority of our patients for male factor infertility suffer from poor semen parameters. For these men, it is difficult to predict whether a single gene defect caused their fertility problem or is more likely the result from an interaction of one or more genes that are potentially reflecting environmental influence.

A number of epidemiological factors that may have a bearing on a couple's fertility include age, though the impact of male age is less obvious than that of the female companion. Smoking by both partners is highly relevant but more evident in males showing that smokers have lower sperm concentrations than nonsmokers [10, 11]. Occupational, environmental, and genetic factors may also be highly relevant. In regard to the latter, there is no doubt that recent advances in assisted conception technology have increased our understanding of the etiology of male infertility, particularly by drawing attention to the major contribution of specific genes [4, 12–16].

Nonetheless, despite advances in the diagnostic work-up of infertile men, in about 50 % of them suffering from impaired spermatogenesis the cause is unrecognized [17–19]. It has been demonstrated that DNA damage in human spermatozoa has been linked with gamete performance, such as poor fertilization, impaired embryo development, increased pregnancy loss, and even possibly some health consequences in the offspring, not excluding cancer [20–23]. However, at this stage the origin and role of sperm DNA fragmentation in the male gamete, as currently measured, is still controversial.

As a consequence, many couples with "unexplained" infertility eventually prove to have defective spermatozoa when appropriately sensitive assays (such as acrosome reaction, antisperm antibodies, and PLCζ) are applied, yet some couples with severely compromised semen parameters prove to have normal sperm function [24, 25].

19.2 Versatility of ICSI

From September 1993 to June 2012, we have performed at Cornell a total of 34,425 ART cycles with an average maternal age for IVF of 37.6±4

Table 19.1 Fertilization and pregnancy rates according to semen origin

	Spermatozoa	
No. of	Ejaculated	Surgically retrieved
Cycles	21,302	2,225
Fertilization (%)	132,183/166,796 (79.2)[a]	12,922/20,779 (62.2)[a]
Clinical pregnancies (%)	8,404 (39.5)[b]	993 (44.6)[b]

[a]χ^2, 2x2, 1 *df*, effect of spermatozoal source on fertilization rate, $P=0.0001$
[b]χ^2, 2x2, 1 *df*, effect of spermatozoal source on clinical pregnancy rate, $P=0.0001$

years and for ICSI of 36.0 ± 5 years, and mean paternal ages of 39.6 ± 6 years and 40.8 ± 8 years, respectively. Of those cycles, 31.7 % (10,898) included the standard in vitro insemination with a fertilization rate of 60.5 % and a clinical pregnancy rate of 37.6 %, the remainder being ICSI cycles.

ICSI was performed in 21,302 cycles with ejaculated spermatozoa with a mean maternal age 36.9 ± 5 years. In our population, over 87% ($n=18,575$) of our men had at least one abnormal semen parameter according to the WHO 2010 criteria. Of the 175,833 MII oocytes injected 5.1 % lysed and those that survived yielded 79.2 % (132,183/166,796) zygotes, 2.5 % of 1PN, and 3.5 % of 3PN (Table 19.1).

When looking at men with severe oligozoospermia ($\leq 1 \times 10^6$/ml) ($n=1,476$), the average sperm concentration was $0.2 \pm 0.3 \times 10^6$/ml, with an average motility of 18.6 ± 20 %, and normal morphology of 0.5 ± 1 %. In these cases, we were able to obtain a fertilization rate of 66.8 % (9,222/13,800) with a clinical pregnancy of 43.3 % (640/1,479).

In cases where no spermatozoa are identified in the counting chamber ($n=188$) and high-speed centrifugation is needed to finally identify sperm cells, yielded an average concentration of $0.008 \pm 0.003 \times 10^3$/ml with a mean motility of 15.8 ± 18 %. Zygote formation occurred in 57.8 % (1,106/1,912) of all oocytes injected with 2.5 embryos transferred per patient providing a clinical pregnancy of 37.8 % (71/188).

In situations where no spermatozoa are found in their ejaculate after two semen analyses, patients opt to undergo epididymal or testicular sperm retrieval. In 2,225 cycles with surgically retrieved spermatozoa, the mean maternal age was 35.1 ± 5 years. A total of 966 cycles were performed with epididymal specimens and 1,259 cycles with testicular samples. When looking at men with obstructive azoospermia that used spermatozoa retrieved from the epididymis, those diagnosed with congenital absence of the vas ($n=524$) had higher fertilization (72.1 % versus 70.9%; $P=0.0001$) as well as clinical pregnancies (54.0 % versus 46.8 %; $p=0.03$) in comparison to those that had an acquired vas obstruction ($n=442$). In cycles that used testicular sampling, we divided them according to their etiology as being obstructive ($n=228$) or nonobstructive ($n=1,031$). In these cases, the fertilization rate was superior in the obstructive cohort when compared to the nonobstructive group (64.5 % versus 52.7 %; $P=0.0001$) but resulting in comparable clinical pregnancies (45.2 % versus 38.8 %).

When the fertilization and pregnancy characteristics were analyzed according to the whether the sample was cryopreserved, we observed that after cryopreservation epididymal samples had lower motility parameters ($p<0.0001$) as well as pregnancy outcome ($p=0.0001$), though without affecting fertilization rate. When testicular samples were used for ICSI, the situation was reversed with zygote formation being higher in the fresh specimens ($p=0.03$) as well as the ability of the embryo to implant ($p=0.0001$) (Table 19.2).

To evaluate differences in performance between insemination methods, we compared embryological outcomes and clinical pregnancy rates between standard in vitro insemination and ICSI. While it appeared that fertilization was lower in IVF than with ICSI ($p=0.0001$; Fig. 19.1), this was confirmed after correcting for

Table 19.2 Spermatozoal parameters and intracytoplasmic sperm injection (ICSI) outcome according to retrieval sites and specimen condition

	Spermatozoa			
	Epididymal		Testicular	
No. of	Fresh	Frozen/thawed	Fresh	Frozen/thawed
Cycles	342	624	917	342
Density (10^6/ml ± SD)	45.8 ± 47	26.6 ± 32	0.4 ± 2	0.2 ± 0.7
Motility (% ± SD)	19.0 ± 17[a]	4.1 ± 8[a]	3.1 ± 7	1.2 ± 4
Morphology (% ± SD)	1.7 ± 2.3	1.2 ± 2	0	0
Fertilization (%)	2,515/3,473 (72.4)	4,104/5,779 (71.0)	4,894/8,568 (57.1)[c]	1,406/2,959 (47.6)[c]
Clinical pregnancies (%)	206 (60.2)[b]	284 (45.5)[b]	382 (41.7)[d]	121 (35.4)[d]

[a]Student's *t*-test, two independent samples, effect of epididymal cryopreservation on sperm motility, $p < 0.0001$
[b]χ^2, 2 × 2, 1 *df*, effect of epididymal cryopreservation on clinical pregnancy rate, $p = 0.0001$
[c, d]χ^2, 2 × 2, 1 *df*, effect of testicular cryopreservation on fertilization and clinical pregnancy rates, $p = 0.0001$

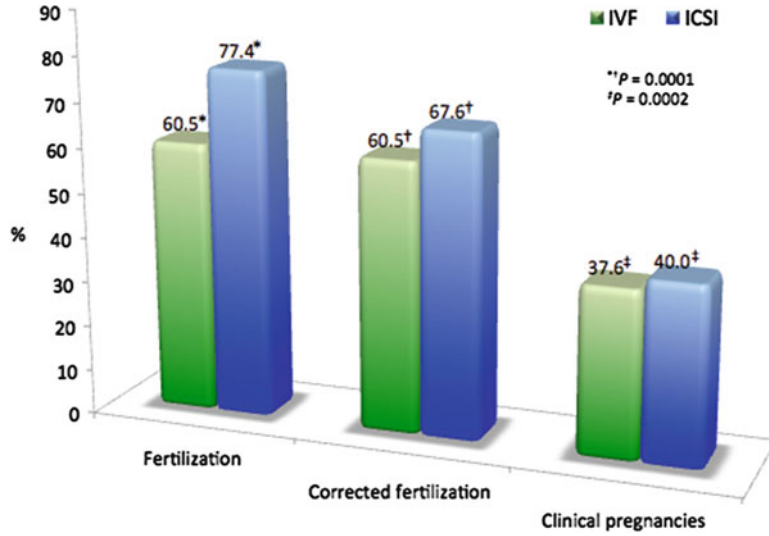

Fig. 19.1 Fertilization and clinical pregnancy reported at Cornell following standard in vitro insemination and ICSI. To better compare fertilization success between the two insemination methods, we have corrected fertilization with ICSI using the total number of oocytes retrieved as the denominator

fertilization for all retrieved oocytes and not for metaphase II, ICSI still yielded more oocytes that fertilized (60.5 % versus 67.6 %; $p = 0.0001$). Furthermore, the ability to generate term pregnancies was also higher with the ICSI cohort ($p = 0.0002$). However, as in all fields of reproductive medicine, the limiting factor remains to be maternal age (Fig. 19.2) as can be evidenced by an inverse relationship between delivery rate and female age.

19.3 Understanding Fertilization

Fertilization is defined as the process resulting from the fusion of the two parental gametes, the egg and the spermatozoon. When mammalian eggs and spermatozoa meet in the oviduct, a series of steps are set in motion that lead to fertilization and ultimately to the development of a new individual. Fertilization induces a cascade of critical

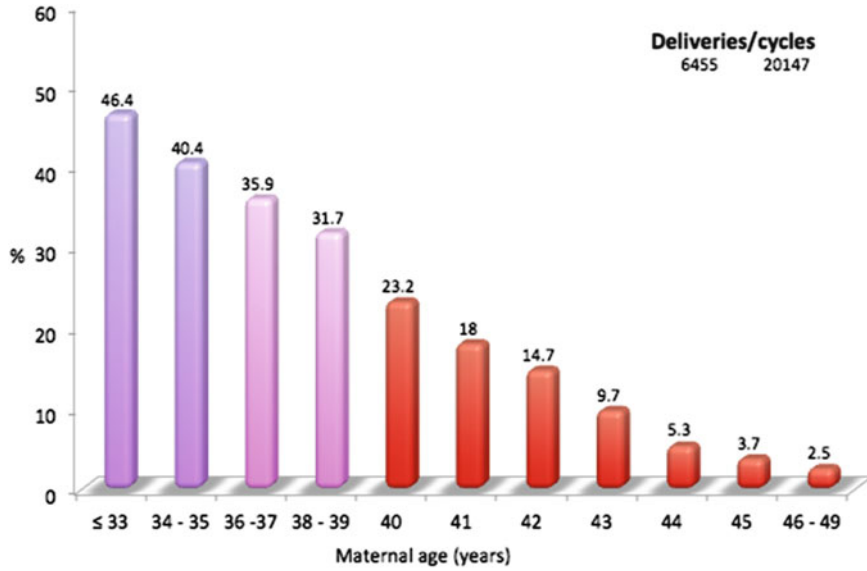

Fig. 19.2 Effect of maternal age in relation to ICSI delivery outcome

events that result in the development of the zygote. Capacitated, free-swimming spermatozoa must initially recognize and bind to the extracellular coat or zona pellucida (ZP) of the ovulated egg. The sperm cell must complete the acrosome reaction that enables it to penetrate through the thickness of the ZP, then must bind to and fuse with the egg plasma membrane to activate the ootid. These multiple steps have been postulated to involve receptor–ligand interactions, ion-channel modulations, membrane fusions, and proteolysis [26–29].

During fertilization, the egg is activated to engage in embryo development. Oocyte activation involves a multitude of molecular changes depending upon the species. Generally, it is triggered by the binding of the male gamete to the oolemma resulting in intracellular Ca^{2+} release within the ooplasm during fertilization [30]. The initial rise of free cytoplasmic Ca^{2+} starts from the site of sperm penetration and expands as a wave through the oocyte [31, 32]. While one Ca^{2+} transient is registered in echinoderm, fish, and frog oocytes [31], repetitive calcium oscillations that last up to several hours are observed in mammals [33, 34]. In mammals the fertilization-dependent Ca^{2+} oscillations were proposed to be due to the release of a soluble cytosolic factor by the sperm following sperm-oolemma fusion [35, 36]. A putative phospholipase released from the sperm head catalyzes the hydrolysis of PIP2 (phosphatidylinositol 4,5-bisphosphate) in the plasma membrane yielding IP_3 (inositol trisphosphate) and DAG (diacylglycerol). IP_3 binds to its receptor present on the endoplasmic reticulum membrane and elicits the flux of Ca^{2+} into the cytoplasm [37] needed to activate the oocyte.

ICSI achieves a fertilization rate between 70 and 80 % with ejaculated spermatozoa independently from the sperm's functionality as long as the male gamete is viable [38]. In some ICSI cases, with a frequency ranging from 3 to 5 % [39], complete fertilization failure occurs [40–44]. This can have various causes, but it most often occurs because of a nucleus-cytoplasmic maturation asynchrony [42, 45–49]. In a proportion of these cases, however, the inability of the male gamete to generate conceptuses depends upon a defect in the presence or function of the sperm cytosolic factor [50, 51].

This is not the only contribution of the spermatozoon that we have learned through ICSI but is also expressed as a structural component that facilitates restoration of diploidy and subsequent

embryonic development requires that each gamete must contain only one half of the diploid complement. The mature oocyte possesses all of the elements necessary for embryonic development but, in humans at least, it lacks an active division center, which originates from the centrosome and is contained within the spermatozoon. Boveri first defined the term "centrosome" as a polar corpuscle containing centrioles [52]. Later it was defined more functionally as a microtubule organizing center (MTOC) [53]. The centrosome in somatic cells is considered to be responsible for two basic events: the nucleation of microtubules and the formulation of an efficient mitotic spindle [54].

In most cells, the centrosome consists of two morphologically distinct centrioles and the pericentriolar material (PCM). Centrioles do not seem to be present in meiotic spindle of gametes but are present at the spindle poles during the first mitotic division in zygotes from various species [55] including humans [56]. The mature human oocyte does not have either centrioles or functional centrosomes associated with its meiotic spindle resulting in anastral, barrel-shaped, and microtubules end abruptly at the poles. The outer pole, however, is closely bound to the egg cortex.

In contrast to the oocyte, the human spermatozoon has two distinct centrioles. A well-defined proximal centriole located within the connecting piece next to the basal plate of the sperm head, displays a 9+0 pattern of nine triplet microtubules surrounded by electron dense material and flanked by nine cross-striated columns. The distal centriole is aligned with the axis of the flagellum perpendicular to the proximal centriole and gives rise to the sperm tail axonome during spermiogenesis [56, 57].

The absence of the sperm centrosome could be one of the causes of embryonic failure [42, 58, 59]. The utilization of biochemical and immunological techniques has now made it possible to identify proteins that are integral components of the centrosome [58, 60–62].

With the occasional exceptions such as the mouse [63], centriolar and centrosomal inheritance in mammals has been assumed to follow a paternal lineage, and there is now little doubt that in humans only the male gamete has an active centrosome [58, 64]. Extensive analysis by transmission electron microscopy (TEM) has demonstrated the presence of centrioles in spermatozoa and in fertilized oocytes at syngamy, and their absence in MII oocytes confirms the paternal inheritance of the centrosome in humans [56]. Furthermore, FISH assessment of chromosome distribution has revealed that the sperm centrosome is solely responsible for organization of the first mitotic division in human embryos [64].

19.4 Safety

Notwithstanding the large number of babies born following ICSI worldwide, concerns still exist as to whether the use of suboptimal spermatozoa can result in genomic or phenotypic abnormalities in the progeny [65]. In one of the earlier studies on the evolution of pregnancies after ICSI, it was observed that the rate of malformation was 2.6 % after ICSI [66]. An extension of the Cornell series, which included a total of 14,211 ART children examined, found that the incidence of overall malformation was comparable between the IVF and ICSI.

These qualms are not only limited to the inheritance of specific traits that bear on fertility, but most importantly those related to the postnatal well-being of the offspring as reflected in growth [67] or cognitive development [68]. Because a complete child development evaluation is labor intensive and costly, we proposed to use a standardized parent–administered questionnaire, Ages and Stages Questionnaire® (ASQ), as an alternative method to evaluate children for developmental delays that are crucial in their first five years of life [69, 70]. In screening a large number of children using the ASQ, we found that the great majority of the 3-year-old children analyzed in the ICSI and IVF groups had normal cognitive abilities, socioemotional development, and motor skill scores [71]. Of the children who had developmental delays, the large majority originated from high-order gestations ($p<0.01$). This further solidifies the theory that single embryo transfer is essential in ensuring a healthy baby.

Interestingly, children whose fathers' gametes were surgically harvested appeared to score better than those conceived with ejaculated spermatozoa by IVF and ICSI [72].

A recent survey on neonates evidenced that ICSI children born after the use of surgically retrieved spermatozoa had, however, a higher incidence of cardiac defects in comparison to ICSI using ejaculated and spontaneously conceived. Another survey completed by parents evidenced that 5- and 7-year-old ART children were more at risk of having respiratory problems characterized as asthma, wheezing, or taking antiasthmatic medication in comparison to children conceived naturally or after ovulation induction [73]. When Leydig cell function of pubertal boys (14 years old) after ICSI was assessed, either through venous puncture or saliva sampling, testosterone concentrations were comparable to naturally conceived boys [74, 75]. In another series of investigations, the same researchers monitored the pubertal development, by Tanner stage (breast, genital, and pubic hair development) and age at menarche, in singleton born ICSI boys and girls, and did not evidence any obvious sexual development in ICSI adolescents in comparison to their 14-year-old spontaneously conceived counterparts except for less pronounced breast development in ICSI females [76]. Finally, adiposity and body fat distribution were also targeted in these 14-year-old adolescents. No differences in body composition measurements were found between ICSI and the control cohort. However, in boys with more advanced pubertal stages, there was a higher sum of peripheral skinfolds. In addition, the peripheral adiposity and body fat percentage of ICSI girls were significantly higher than their spontaneously conceived counterpart [77].

Overall, studies of children ranging from newborn to 14 years of age [73, 74, 76, 78–82] have been reassuring in terms of perinatal outcome, IQ, and physical development [83]. Further follow-up on ICSI teenagers into adulthood should be continued to better understand the reproductive capacity of these youngsters.

The specific concerns in regard to ICSI, whether real or theoretical [84–87], involve the insemination method, the use of spermatozoa with genetic or structural defects, and the possible introduction of foreign genes. Several epidemiological studies of assisted reproduction children report a twofold increase in infant malformations [88], a recurrent reduction in birth weight [89], certain rare syndromes related to imprinting errors [90–94], and even a higher frequency of some cancers [95]. However, such observations do not prove that there is an increased risk of imprinting disorders and even less so childhood cancers in ICSI children [87].

Thus far, Beckwith–Wiedemann Syndrome (BWS) is the only epigenetic disorder that has been clearly associated with ART procedures [96] and has been found to be equally distributed among the in vitro conception methods. Epigenetic imbalances have been similarly linked to the exposure of the embryos to long-term culture [83, 97]. At present, there is no evidence that the ICSI insemination itself is responsible for any increase in epigenetic disorders, findings that have been confirmed in animal studies [98].

In summary, the most important factor that can lead to adverse outcomes in offspring conceived by IVF or ICSI is the occurrence of high-order pregnancies. However, the introduction of single embryo transfer has reduced this considerably. Although perinatal outcomes such as prematurity, low birth weight, perinatal mortality, and increased incidence of malformations have been linked to the techniques of IVF and ICSI, the main culprit seems related to infertility itself. Overall, no significant long-term neurodevelopmental differences have been found in connection with the ARTs, though the risks associated with childhood cancer and future fertility still require further investigation.

19.5 So, Why ICSI?

We have listed the often indistinct indications of a couple's infertility with many of them attributable to the male partner. In our current practice once the ART option is offered to a couple, it may be intricate to choose a specific method of insemination because of the looming chances of failed

fertilization for subtle factors conferred by both gametes. This pressure on the infertility specialist may induce them to bypass the academic approach and directly target a microinsemination method. ICSI would spare the couple emotional and financial tolls related to the unforeseen fertilization failure. While ICSI was born to address specific defects of the male gamete, it is now mastered worldwide and aimed at an array of applications.

ICSI has been equally successful whether the sperm sample is fresh or frozen, is unrelated to the characteristics of the semen parameters or unaffected to the presence of anti-sperm antibodies. Similarly, whether spermatozoa are ejaculated or retrieved surgically from the epididymis or testis, appear not to be a factor weighing on the outcome. Moreover, ICSI's dependability has broadened its initial use from a technique capable of overriding the dysfunctionality of spermatozoa to one that may partly compensate for problems with the egg. Indeed, ICSI has allowed successful fertilization when only few and/or abnormal oocytes were available. Stripping cumulus cells off the oocytes allows a direct visualization of oocyte maturation occurrence, therefore offering a woman with a limited number of oocytes a much greater chance of successful fertilization. In fact, the availability of ICSI has been instrumental in some European countries that include Italy and Germany in circumventing restrictive legislation that limits the number of oocytes inseminated or embryos to be replaced.

ICSI has also made the consistent fertilization of cryopreserved oocytes possible where premature exocytosis of cortical granules causes zona hardening, thereby inhibiting natural sperm penetration. Furthermore, ICSI is the preferred conception method during the application of preimplantation genetic diagnosis by avoiding sperm DNA zona contamination and enhancing the number of embryos available for screening.

Moreover, ICSI has an impact in the arena of HIV infection. Many patients infected with HIV-1 show interests in beginning a family, most serodiscordant couples are concerned, nevertheless, with the possibility of both horizontal and vertical transmission of the virus. In such cases, intrauterine insemination with spermatozoa processed by double-gradient centrifugation followed by swim up has been the suggested method of treating serodiscordant couples with an HIV-1 infected male partner. The use of ICSI has been proposed by several groups because of its negligible oocyte exposure to semen, thereby reducing the risk of viral transmission.

We can in fact use all spermatozoa collected from different sources within the male reproductive system and we can be nonchalant about the presence of antisperm antibodies, careless about the sperm preparation, even callous about its morphology and if the spermatozoa has an acrosome or even an abnormal chromatin packing, a complete flagellum or display motility. These applications for ICSI seem destined only to increase even more with the surfacing of streamlined and automated approaches being developed to carry out in vitro insemination.

It is because of the ICSI practice that we reap the most benefits related to this entirely different way of generating a conceptus. In fact, the removal of the cumulus cells to allow injection offers a window of assessing the maturity of oocytes and, therefore, pinpoint the exact timing of when fertilization begins while providing information on the efficacy of an ovarian stimulation, particularly nowadays when we are trying to reduce the stress to the ovary and minimize the oocyte retrieved while improving their quality. The abnormal ICSI fertilization has helped us to shed light on the inheritance of the centrosome as previously elucidated.

ICSI has been invaluable in understanding the mechanism of oocyte activation whether due to ooplasmic asynchrony or a lack of spermatozoal cytosolic factor. Nonetheless, the use of ICSI has generated a great deal of concern since it may increase the risk of transmitting genetics diseases to the offspring as confirmed by studies reporting on the higher incidence of sex chromosomal aneuploidy of paternal origin and structural de novo chromosomal abnormalities in children conceived after ICSI, compared to the general population. This indicated that spermatogenic impairment due to a primitive testiculopathy have an increased sperm aneuploidy rate, which negatively correlates

with the main sperm parameters despite a normal somatic karyotype. This suggests that the *noxae* acting at the testicular level not only impairs spermatogenesis but affects the molecular mechanisms involved in chromosomal segregation.

In spite of these unsettling implications, the well-being of ICSI offspring is reassuring as long as a singleton term pregnancy is achieved. This is also true when surgically samples or when scarce sperm cells with "prohibitive" morphology have been injected.

19.6 Conclusions

The take-home message from this presentation is that during consultation with infertile couples presenting with borderline semen parameters, where there are dubious chances of fertilizing a conceptus, or when it is foreseen that the individuals may not be able to emotionally handle an unexpected fertilization failure, ICSI should not be denied. As in all aspects of medicine, the counseling begins with the description of the side effects of medications, the surgical risks linked to egg retrieval, the possibility to transmit genetic disorders, even if currently unknown to the prospective parents, related to the oocyte or the sperm. Nonetheless, the chances of a de novo appearance of a disorder resulting from the syngamy of the two parental gametes should be contemplated. The concerns related to the health of the ICSI offspring are not linked to the procedure itself but mostly to the genetic or epigenetic conditions of the parents that is expressed through the utilization of their gametes.

References

1. Collins JA, Milner RA, Rowe TC. The effect of treatment on pregnancy among couples with unexplained infertility. Int J Fertil. 1991;36(3):140–1.
2. CDC RH. Assisted reproductive technology success rates. National summary and fertility Clinic reports. National Center for Chronic Disease and Prevention and Health Promotion. 2011 November 2011.
3. WHO. WHO laboratory manual for the examination and processing of human semen. 5th ed. Cambridge: Cambridge University Press; 2010.
4. Reijo R, Lee TY, Salo P, et al. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat Genet. 1995;10(4):383–93.
5. Matthews GJ, Matthews ED, Goldstein M. Induction of spermatogenesis and achievement of pregnancy after microsurgical varicocelectomy in men with azoospermia and severe oligoasthenospermia. Fertil Steril. 1998;70(1):71–5.
6. Ferlin A, Raicu F, Gatta V, et al. Male infertility: role of genetic background. Reprod Biomed Online. 2007;14(6):734–45.
7. Massart A, Lissens W, Tournaye H, et al. Genetic causes of spermatogenic failure. Asian J Androl. 2012;14(1):40–8.
8. Dam AH, Koscinski I, Kremer JA, et al. Homozygous mutation in SPATA16 is associated with male infertility in human globozoospermia. Am J Hum Genet. 2007;81(4):813–20.
9. Liu G, Shi QW, Lu GX. A newly discovered mutation in PICK1 in a human with globozoospermia. Asian J Androl. 2010;12(4):556–60.
10. Joffe M, Li Z. Association of time to pregnancy and the outcome of pregnancy. Fertil Steril. 1994; 62(1):71–5.
11. Vine MF. Worldwide decline in semen quality might be due to smoking. BMJ. 1996;312(7029):506.
12. Kent-First MG, Kol S, Muallem A, et al. Infertility in intracytoplasmic-sperm-injection-derived sons. Lancet. 1996;348(9023):332.
13. McLachlan RI, Mallidis C, Ma K, et al. Genetic disorders and spermatogenesis. Reprod Fertil Dev. 1998;10(1):97–104.
14. Repping S, Skaletsky H, Lange J, et al. Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. Am J Hum Genet. 2002;71(4):906–22.
15. Simoni M, Gromoll J, Dworniczak B, et al. Screening for deletions of the Y chromosome involving the DAZ (Deleted in AZoospermia) gene in azoospermia and severe oligozoospermia. Fertil Steril. 1997;67(3): 542–7.
16. Vogt PH, Edelmann A, Kirsch S, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Hum Mol Genet. 1996; 5(7):933–43.
17. Dohle GR, Colpi GM, Hargreave TB, et al. EAU guidelines on male infertility. Eur Urol. 2005;48(5): 703–11.
18. Forti G, Krausz C. Clinical review 100: Evaluation and treatment of the infertile couple. J Clin Endocrinol Metab. 1998;83(12):4177–88.
19. Guzick DS, Overstreet JW, Factor-Litvak P, et al. Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med. 2001;345(19): 1388–93.
20. Borini A, Tarozzi N, Bizzaro D, et al. Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Hum Reprod. 2006;21(11):2876–81.

21. Cohen-Bacrie P, Belloc S, Menezo YJ, et al. Correlation between DNA damage and sperm parameters: a prospective study of 1,633 patients. Fertil Steril. 2009;91(5):1801–5.
22. Ji BT, Shu XO, Linet MS, et al. Paternal cigarette smoking and the risk of childhood cancer among offspring of nonsmoking mothers. J Natl Cancer Inst. 1997;89(3):238–44.
23. Lewis SE, Aitken RJ. DNA damage to spermatozoa has impacts on fertilization and pregnancy. Cell Tissue Res. 2005;322(1):33–41.
24. Glazener CM, Coulson C, Lambert PA, et al. The value of artificial insemination with husband's semen in infertility due to failure of postcoital sperm-mucus penetration–controlled trial of treatment. Br J Obstet Gynaecol. 1987;94(8):774–8.
25. Irvine DS, Aitken RJ, Lees MM, et al. Failure of high intrauterine insemination of husband's semen. Lancet. 1986;2(8513):972–3.
26. Wassarman PM, Jovine L, Qi H, et al. Recent aspects of mammalian fertilization research. Mol Cell Endocrinol. 2005;234(1–2):95–103.
27. Hardy K, Wright C, Rice S, et al. Future developments in assisted reproduction in humans. Reproduction. 2002;123(2):171–83.
28. Sutovsky P. Sperm-egg adhesion and fusion in mammals. Expert Rev Mol Med. 2009;11:e11.
29. Nomikos M, Elgmati K, Theodoridou M, et al. Phospholipase Czeta binding to PtdIns(4,5)P2 requires the XY-linker region. J Cell Sci. 2011;124(Pt 15): 2582–90.
30. Lawrence Y, Whitaker M, Swann K. Sperm-egg fusion is the prelude to the initial Ca^{2+} increase at fertilization in the mouse. Development. 1997;124(1): 233–41.
31. Jaffe LA, Cross NL, Picheral B. Studies of the voltage-dependent polyspermy block using cross-species fertilization of amphibians. Dev Biol. 1983;98(2): 319–26.
32. Swann K. The soluble sperm oscillogen hypothesis. Zygote. 1993;1(4):273–6.
33. Miyazaki S, Shirakawa H, Nakada K, et al. Essential role of the inositol 1,4,5-trisphosphate receptor/Ca^{2+} release channel in Ca^{2+} waves and Ca^{2+} oscillations at fertilization of mammalian eggs. Dev Biol. 1993; 158(1):62–78.
34. Sun DS, Lo SJ, Lin CH, et al. Calcium oscillation and phosphatidylinositol 3-kinase positively regulate integrin alpha(IIb)beta3-mediated outside-in signaling. J Biomed Sci. 2005;12(2):321–33.
35. Dale B, DeFelice LJ, Ehrenstein G. Injection of a soluble sperm fraction into sea-urchin eggs triggers the cortical reaction. Experientia. 1985;41(8):1068–70.
36. Swann K. A cytosolic sperm factor stimulates repetitive calcium increases and mimics fertilization in hamster eggs. Development. 1990;110(4):1295–302.
37. Berridge MJ. Inositol trisphosphate and calcium signalling. Nature. 1993;361(6410):315–25.
38. Palermo GD, Neri QV, Takeuchi T, et al. ICSI: where we have been and where we are going. Seminars Reprod Med. 2009;27(2):191–201.
39. Esfandiari N, Faved MH, Gotlieb L, et al. Complete failed fertilization after intracytoplasmic sperm injection—Analysis of 10 years' data. Int J Fertil Womens Med. 2005;50(4):187–92.
40. Devroey P, Liu J, Nagy Z, et al. Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. Hum Reprod. 1995;10(6):1457–60.
41. Flaherty SP, Payne D, Swann NJ, et al. Aetiology of failed and abnormal fertilization after intracytoplasmic sperm injection. Hum Reprod. 1995; 10(10):2623–9.
42. Moomjy M, Colombero LT, Veeck LL, et al. Sperm integrity is critical for normal mitotic division and early embryonic development. Mol Hum Reprod. 1999;5(9):836–44.
43. Palermo GD, Schlegel PN, Colombero LT, et al. Aggressive sperm immobilization prior to intracytoplasmic sperm injection with immature spermatozoa improves fertilization and pregnancy rates. Hum Reprod. 1996;11(5):1023–9.
44. Sousa M, Tesarik J. Ultrastructural analysis of fertilization failure after intracytoplasmic sperm injection. Hum Reprod. 1994;9(12):2374–80.
45. Kang H, Neri QV, Takeuchi T, et al. Investigating unexpected complete fertilization failure with ICSI. Fertil Steril. 2005;84(1 Suppl):S257.
46. Kocent J, Monahan D, Neri QV, et al. Untimely cumulus removal affects ooplasmic competence. Fertil Steril. 2010;94(4 Suppl):S252.
47. Neri QV, Lee B, Rosenwaks Z, et al. Understanding fertilization through intracytoplasmic sperm injection (ICSI). Cell Calcium. being reviewed.
48. Palermo GD, Neri QV, Monahan D, et al. Development and current applications of assisted fertilization. Fertil Steril. 2012;97:248–59.
49. Sundstrom P, Nilsson BO. Meiotic and cytoplasmic maturation of oocytes collected in stimulated cycles is asynchronous. Hum Reprod. 1988;3(5):613–9.
50. Kashir J, Heindryckx B, Jones C, et al. Oocyte activation, phospholipase C zeta and human infertility. Hum Reprod Update. 2010;16(6):690–703.
51. Swann K, Larman MG, Saunders CM, et al. The cytosolic sperm factor that triggers Ca^{2+} oscillations and egg activation in mammals is a novel phospholipase C: PLC zeta. Reproduction. 2004;127(4):431–9.
52. Boveri T. Ueber die "Befruchtung der Eier von Ascaris megalocephala". Sitz-Ber Ges Morph Phys Mçnchen. 1887;3:394–443.
53. Pickett-Heaps J, Spurck T, Tippit D. Chromosome motion and the spindle matrix. J Cell Biol. 1984;99(1 Pt 2):137s–43s.
54. Bornens M, Paintrand M, Berges J, et al. Structural and chemical characterization of isolated centrosomes. Cell Motil Cytoskeleton. 1987;8(3):238–49.
55. Le Guen P, Crozet N. Microtubule and centrosome distribution during sheep fertilization. Eur J Cell Biol. 1989;48(2):239–49.
56. Sathananthan AH, Kola I, Osborne J, et al. Centrioles in the beginning of human development. Proc Natl Acad Sci USA. 1991;88(11):4806–10.

57. Sathananthan AH, Ratnam SS, Ng SC, et al. The sperm centriole: its inheritance, replication and perpetuation in early human embryos. Hum Reprod. 1996;11(2):345–56.
58. Palermo GD, Avrech OM, Colombero LT, et al. Human sperm cytosolic factor triggers Ca^{2+} oscillations and overcomes activation failure of mammalian oocytes. Mol Hum Reprod. 1997;3(4):367–74.
59. Van Blerkom J, Davis P. Evolution of the sperm aster after microinjection of isolated human sperm centrosomes into meiotically mature human oocytes. Hum Reprod. 1995;10(8):2179–82.
60. Colombero LT, Moomjy M, Sills ES, et al. The role of structural integrity of the fertilising spermatozoon in early human embryogenesis. Zygote. 1999;7(2):157–63.
61. Kimble M, Kuriyama R. Functional components of microtubule-organizing centers. Int Rev Cytol. 1992;136:1–50.
62. Neri QV, Scala V, Rosenwaks Z, et al. Assessment of the sperm centrosome. Fertil Steril. 2011;96(3 Suppl 1): S235–6.
63. Schatten G, Simerly C, Schatten H. Maternal inheritance of centrosomes in mammals? Studies on parthenogenesis and polyspermy in mice. Proc Natl Acad Sci USA. 1991;88(15):6785–9.
64. Palermo G, Munne S, Cohen J. The human zygote inherits its mitotic potential from the male gamete. Hum Reprod. 1994;9(7):1220–5.
65. Ludwig M, Katalinic A. Malformation rate in fetuses and children conceived after ICSI: results of a prospective cohort study. Reprod Biomed Online. 2002;5(2):171–8.
66. Palermo GD, Colombero LT, Schattman GL, et al. Evolution of pregnancies and initial follow-up of newborns delivered after intracytoplasmic sperm injection. JAMA. 1996;276(23):1893–7.
67. Schieve LA, Rasmussen SA, Buck GM, et al. Are children born after assisted reproductive technology at increased risk for adverse health outcomes? Obstet Gynecol. 2004;103(6):1154–63.
68. Bowen JR, Gibson FL, Leslie GI, et al. Medical and developmental outcome at 1 year for children conceived by intracytoplasmic sperm injection. Lancet. 1998;351(9115):1529–34.
69. Squires J, Carter A, Kaplan P. Developmental monitoring of children conceived by intracytoplasmic sperm injection and in vitro fertilization. Fertil Steril. 2003;79(2):453–4.
70. Neri QV, Tanaka N, Wang A, et al. Intracytoplasmic sperm injection. Accomplishments and qualms. Minerva Ginecol. 2004;56(3):189–96.
71. Palermo GD, Neri QV, Takeuchi T, et al. Genetic and epigenetic characteristics of ICSI children. Reprod Biomed Online. 2008;17(6):820–33.
72. Neri QV, Williams-Pitman M, Rosenwaks Z, et al. Cognitive and behavioral profiles of ICSI children conceived from surgically retrieved spermatozoa. Fertil Steril. 2007;88 Suppl 1:S39.
73. Carson C, Sacker A, Kelly Y, et al. Asthma in children born after infertility treatment: findings from the UK Millennium Cohort Study. Hum Reprod. 2013;28(2): 471–9.
74. Belva F, Bonduelle M, Schiettecatte J, et al. Salivary testosterone concentrations in pubertal ICSI boys compared with spontaneously conceived boys. Hum Reprod. 2011;26(2):438–41.
75. Belva F, Bonduelle M, Painter RC, et al. Serum inhibin B concentrations in pubertal boys conceived by ICSI: first results. Hum Reprod. 2010;25(11): 2811–4.
76. Belva F, Roelants M, Painter R, et al. Pubertal development in ICSI children. Hum Reprod. 2012;27(4): 1156–61.
77. Belva F, Painter R, Bonduelle M, et al. Are ICSI adolescents at risk for increased adiposity? Hum Reprod. 2012;27(1):257–64.
78. Belva F, Henriet S, Liebaers I, et al. Medical outcome of 8-year-old singleton ICSI children (born >or=32 weeks' gestation) and a spontaneously conceived comparison group. Hum Reprod. 2007;22(2):506–15.
79. Knoester M, Helmerhorst FM, Vandenbroucke JP, et al. Perinatal outcome, health, growth, and medical care utilization of 5- to 8-year-old intracytoplasmic sperm injection singletons. Fertil Steril. 2008;89(5):1133–46.
80. Leunens L, Celestin-Westreich S, Bonduelle M, et al. Follow-up of cognitive and motor development of 10-year-old singleton children born after ICSI compared with spontaneously conceived children. Hum Reprod. 2008;23(1):105–11.
81. Goldbeck L, Gagsteiger F, Mindermann I, et al. Cognitive development of singletons conceived by intracytoplasmic sperm injection or in vitro fertilization at age 5 and 10 years. J Pediatr Psychol. 2009;34(7):774–81.
82. Basatemur E, Shevlin M, Sutcliffe A. Growth of children conceived by IVF and ICSI up to 12years of age. Reprod Biomed Online. 2010;20(1):144–9.
83. Basatemur E, Sutcliffe A. Follow-up of children born after ART. Placenta. 2008;29(Suppl B):135–40.
84. Cummins JM, Jequier AM. Treating male infertility needs more clinical andrology, not less. Hum Reprod. 1994;9(7):1214–9.
85. de Kretser DM. The potential of intracytoplasmic sperm injection (ICSI) to transmit genetic defects causing male infertility. Reprod Fertil Dev. 1995;7(2):137–41. discussion 41–2.
86. De Rycke M, Liebaers I, Van Steirteghem A. Epigenetic risks related to assisted reproductive technologies: risk analysis and epigenetic inheritance. Hum Reprod. 2002;17(10):2487–94.
87. Edwards RG, Ludwig M. Are major defects in children conceived in vitro due to innate problems in patients or to induced genetic damage? Reprod Biomed Online. 2003;7(2):131–8.
88. Hansen M, Kurinczuk JJ, Bower C, et al. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. N Engl J Med. 2002;346(10):725–30.
89. Schieve LA, Meikle SF, Ferre C, et al. Low and very low birth weight in infants conceived with use of

assisted reproductive technology. N Engl J Med. 2002;346(10):731–7.
90. DeBaun MR, Niemitz EL, Feinberg AP. Association of in vitro fertilization with Beckwith–Wiedemann syndrome and epigenetic alterations of LIT1 and H19. Am J Hum Genet. 2003;72(1):156–60.
91. Gicquel C, Gaston V, Mandelbaum J, et al. In vitro fertilization may increase the risk of Beckwith–Wiedemann syndrome related to the abnormal imprinting of the KCN1OT gene. Am J Hum Genet. 2003;72(5):1338–41.
92. Maher ER, Brueton LA, Bowdin SC, et al. Beckwith–Wiedemann syndrome and assisted reproduction technology (ART). J Med Genet. 2003;40(1):62–4.
93. Orstavik KH. [Intracytoplasmic sperm injection and congenital syndromes because of imprinting defects]. Tidsskr Nor Laegeforen. 2003;123(2):177.
94. Halliday J, Oke K, Breheny S, et al. Beckwith–Wiedemann syndrome and IVF: a case–control study. Am J Hum Genet. 2004;75(3):526–8.
95. Moll AC, Imhof SM, Schouten-van Meeteren AY, et al. In-vitro fertilisation and retinoblastoma. Lancet. 2003;361(9366):1392.
96. Sutcliffe AG, Peters CJ, Bowdin S, et al. Assisted reproductive therapies and imprinting disorders–a preliminary British survey. Hum Reprod. 2006;21(4):1009–11.
97. Rivera RM, Stein P, Weaver JR, et al. Manipulations of mouse embryos prior to implantation result in aberrant expression of imprinted genes on day 9.5 of development. Hum Mol Genet. 2008;17(1):1–14.
98. Wilson TJ, Lacham-Kaplan O, Gould J, et al. Comparison of mice born after intracytoplasmic sperm injection with in vitro fertilization and natural mating. Mol Reprod Dev. 2007;74(4):512–9.

Part V

Clinical Research Design

Cohort Designs: Critical Considerations for Reproductive Health

20

Stacey A. Missmer and Germaine M. Buck Louis

20.1 Introduction

Reproductive health has historically focused on maternal and child health. This focus led to a rather narrow definition that implicitly denoted pregnancy and its related outcomes. More inclusive definitions have evolved in recognition of the many facets encompassing reproductive health, particularly from a population health perspective. For purposes of this chapter, we utilize the definition of reproductive health that was established at the International Conference on Population and Development as, "… a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity, in all matters relating to the reproductive system…" [1]. This definition gives way to a spectrum of reproductive health endpoints and related impairments suitable for clinical and population health research, including research involving (pre)adolescents, men, women, and couples.

From a research perspective, reproductive health encompasses two broad domains—fecundity and fertility—each of which has an associated spectrum of interrelated endpoints. Fecundity is defined as the biologic capacity of men and women for reproduction irrespective of pregnancy intentions, while fertility denotes live birth [2]. As such, fecundity is a necessary but *not* sufficient "cause" of fertility. Clinicians and researchers spend considerable time focusing on fecundity- and fertility-related impairments, in light of the many lingering data gaps underlying "normal" human reproduction and development that preclude a more complete understanding of the pathophysiology of reproductive impairments. Figure 20.1a illustrates our research paradigm for reproductive health encompassing the two domains to emphasize the spectrum of endpoints suitable for study in both men and women, all of which are well suited to prospective design. It also reflects the many opportunities for exposures to alter interrelated endpoints, which may be responsible for the inefficient nature of human reproduction relative to other species, as characterized by low cycle specific conception probabilities, inefficient spermatogenesis, and high rates of pregnancy loss [3–7]. In addition, Fig. 20.1b is a modification of this

S.A. Missmer, Sc.D. (✉)
Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women's Hospital,
Harvard Schools of Medicine and Public Health,
221 Longwood Ave, Boston, MA 02115, USA
e-mail: stacey.missmer@channing.harvard.edu

G.M.B. Louis, Ph.D., M.S.
Division of Epidemiology, Statistics and Prevention Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development,
Rockville, MD, USA
e-mail: louisg@mail.nih.gov

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5_20,

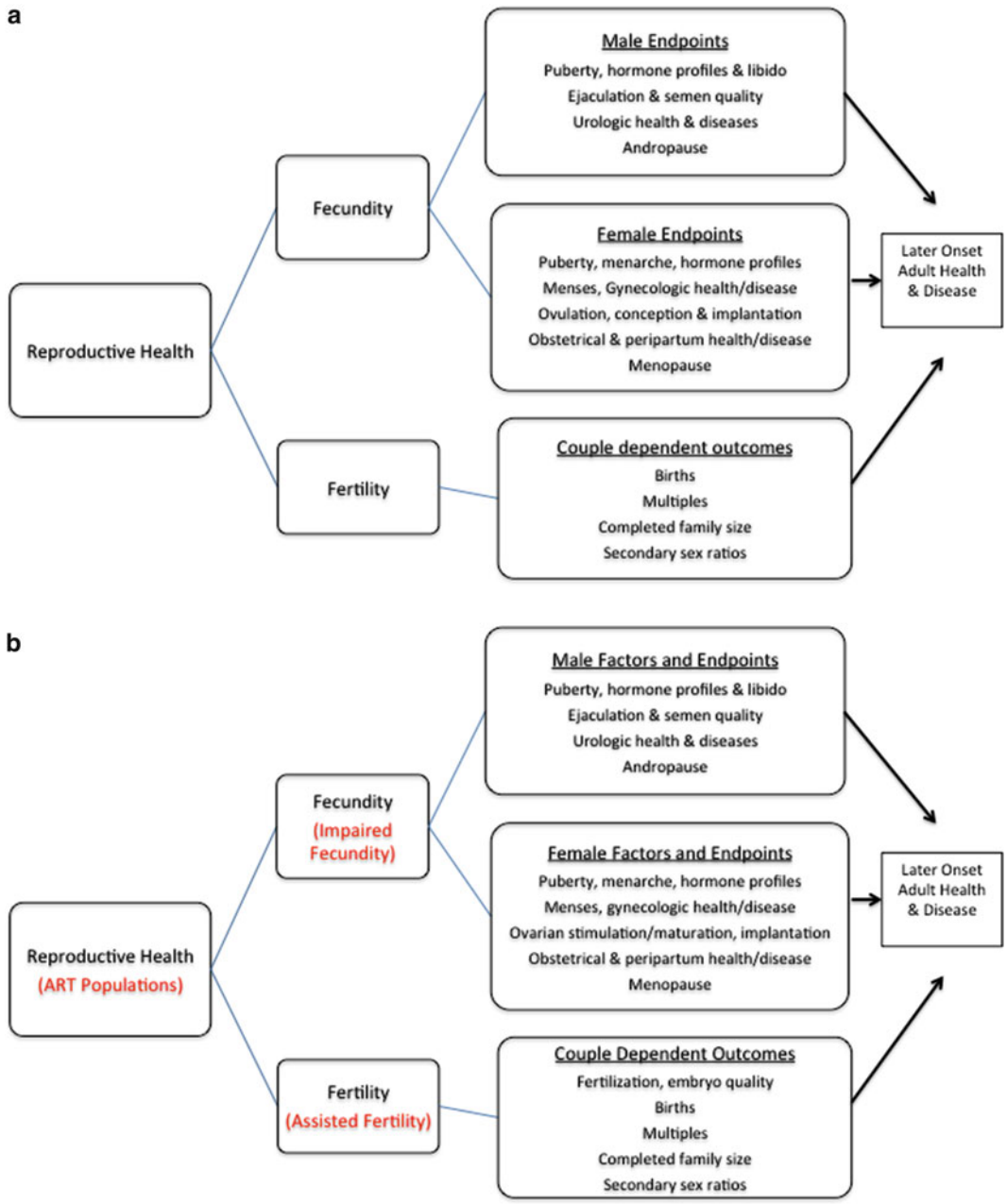

Fig. 20.1 (**a**) General population research paradigm for reproductive health suitable for cohort research designs. (**b**) Research paradigm for reproductive health among subfecund ART populations suitable for cohort research designs

general population paradigm, specific to the population utilizing assisted reproductive technologies (ART). This population is particularly well suited to the cohort design—whether observational or interventional (i.e., the randomized controlled trial) with the exposure assigned, given the short duration from treatment commencement to evidence of an outcome.

20.2 Conceptual and Methodologic Challenges

The conceptual paradigm for reproductive health as presented in Fig. 20.1a, b needs to be operationalized for research purposes to accommodate many unique aspects of successful human reproduction and development. These include: determining the targeted referent and study populations, selection of the appropriate sampling framework for establishing the cohort, and designing data collection and analytic approaches that can reflect temporality, capture the hierarchical exposure data structure and either the hidden or correlated nature of many reproductive study outcomes.

One of the unique challenges underlying study of fecundity-related reproductive health outcomes is the absence of sampling frameworks for identifying and recruiting reproductive aged men and women who are representative of their referent populations. For example, investigators interested in prospective study of the environmental (nongenetic) determinants of spermatogenesis or menstruation do not have a priori defined sampling frameworks available for study. There is no registry, per se, comprising pubescent males or those aged ≥18 years with the possible exception of the selective military services registries in many countries. Motor vehicle driving registries are another possible sampling framework for reproductive aged populations, given their high population prevalence for legal driving or proof-of-identity licenses. However, such registries are often not available for research purposes and conditions for use vary across States.

Sampling frameworks become even more challenging for specific research questions, such as in time-to-pregnancy (TTP) studies, which estimates couple fecundity defined as the time (menstrual cycles or calendar months) required to become pregnant. An inherent complexity is that approximately 38 % of pregnancies in every U.S. State are unintended (defined as either mistimed or unwanted), and more than half are unintended in 29 States and the District of Columbia [8]. By definition, only the proportion of couples actively planning conception is among the eligible population; however, they may not represent the full distribution of exposures or reproductive health. Such designs using the gold standard of preconception recruitment face even more sampling challenges stemming from the narrow interval between discontinuing contraception and the beginning of the trying attempt. Two recent population-based sampling attempts to recruit couples for fecundity studies have reported that ≤1 % of reproductive aged couples may be planning or at risk for pregnancy at any point in time [26, 27], underscoring the need for large sampling frameworks for population research. Table 20.1 lists some of the sampling frameworks utilized by researchers for assessing fecundity (e.g., TTP) or its impairments (e.g., early pregnancy loss), including convenience- and population-based studies. Women, and to a lesser extent couples, have been recruited through traditional media sources and most recently through the internet [22], and from established registries ranging from health insurance to occupation and regulatory registries. Lastly, it is imperative that the sampling unit (e.g., women,

Table 20.1 Existing research focusing on couple fecundity as measured by time-to-pregnancy or early pregnancy loss by type of sampling framework

Convenience sampling (authors)	Population-based sampling (authors)
Volunteers	
Miller et al. (1980) [9] (early pregnancy loss)	*Motor vehicle registry*
Whitaker et al. (1983) [10] (early pregnancy loss)	Sweeney et al. (1989) [11]
France et al. (1984) [12]	Ellish et al. (1996) [13]
De Mouzon et al. (1988[a]) [14]	
Wilcox et al. (1988) [15]	*Occupational cohorts*
Vartiainen et al. (1994) [16]	Hakim et al. (1995) [17]
Zinaman et al. (1996[a]) [18]	Bonde et al. (1998[a]) [3]
Columbo et al. (2000[a]) [19]	Wang et al. (2003) [20]
Pyper et al. (2004) [21]	
Mikkelsen et al. (2009) [22]	*Health maintenance organization*
	Brown et al. (1996) [23]
	Fish/hunting license registries
	Buck et al. (2002) [24]
	Buck Louis et al. (2011[a]) [25]

[a]Recruited couples not just women

couples, and menstrual cycle) be specified at the design phase so that the appropriate analytic plan can be designed and implemented.

Once the study population and sampling framework have been established as determined by the research question and other logical considerations, the inherent methodologic nuances underlying human reproduction and development need to be considered. These include (1) recognition of the endogenous and exogenous nature of exposures relevant for reproductive health; (2) hidden outcome data; (3) hierarchical exposure data structure; (4) clustering of many study outcomes; and (5) model specification responsive to censoring and missingness of data. These are particularly complex issues within the context of ART, where multiple cycles can be attempted, intervention during subsequent cycles is informed by the clustered outcomes observed in the previous cycle, and censoring is typically correlated with both exposure factors and outcome probabilities [28, 29]. Collectively, these methodologic aspects of scientifically rigorous research impact the interpretation of research findings. A brief description of each follows as they pertain to prospective inquiry, though other resources exist for a more complete description of the issues [30–33].

Many factors of interest for reproductive health can be both endogenous and exogenous in nature. Stress is such an example as women with higher concentrations of alpha amylase, a salivary stress biomarker, are reported to have a lower probability of conception each day during the fertile window relative to women with lower concentrations [25]. Thus, stress can be an endogenous factor leading to diminished libido, which may increase TTP leading to even higher exogenous stress levels when pregnancy fails to occur. The hidden data issue reflects the longstanding recognition that many reproductive health outcomes cannot be measured [34], with the exception of unique population subgroups such as couples undergoing assisted reproductive technologies, where otherwise unobservable intermediate outcomes such as ovarian response, fertilization, and embryo quality can be captured. Such hidden data or outcomes include ovulation, conception, implantation, and very early pregnancy loss. Clinical subgroups (e.g., ART) of the population comprise an excellent sampling framework for capturing to the extent possible hidden outcomes, particularly if probability based rather than convenience based sampling strategies are utilized. A hierarchical data structure is a unique hallmark of fecundity and fertility endpoints and has the added challenge of pertaining not only to the male or female but also to the couple if couples are the sampling unit. Figure 20.2 illustrates the hierarchical data structure for cohort studies, particularly for couple-dependent outcomes such as TTP, conception, or pregnancy. Data collection instruments need to be designed to capture the anticipated hierarchical structure.

Correlated or clustered outcomes are nonindependent and they require appropriate techniques for analysis. Clustered outcomes can include both nonindependent observations in groups or repeated observations for the same individual. Examples of correlated outcomes include multiple or higher order births, multiple semen samples per male, multiple menstrual cycles per female, multiple infertility treatment cycles per couple, or multiple embryos within a single infertility treatment cycle. The repeatability of fecundity (e.g., TTP) as well as impaired fecundity (e.g., pregnancy loss) is well known [35–37]. This longstanding clinical observation has led to prior history of an adverse pregnancy outcome being considered a risk factor for subsequent pregnancies in many perinatal scoring systems. Historically, investigators have either ignored such clustering or designed it away by restricting analysis to one treatment cycle or pregnancy per woman. Correlated outcomes are a key consideration for assisted reproductive technologies, given that many couples have multiple treatment cycles accompanied by a varying number of oocytes retrieved or embryos created and transferred per cycle [28]. Of note, failure to account for any correlated outcomes may lead to unpredictable bias or incorrect conclusions [28, 38–40]. Fortunately, several new modeling strategies are available to deal with the complex within-cluster correlation such dependency introduces. These approaches include hierarchical models such as

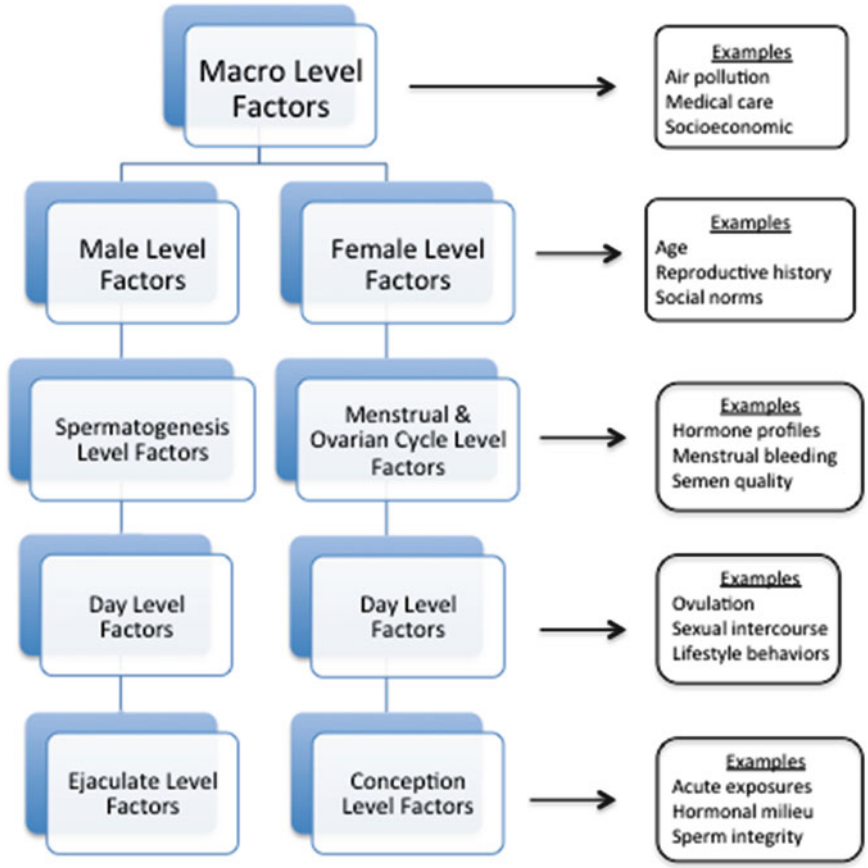

Fig. 20.2 Illustration of the hierarchical data structure for various reproductive health outcomes

Bayesian methods, mixed models, or generalized estimating equations (GEE). However, GEE analysis estimates only population-level not subject-specific inference, the latter which is most relevant for clinical prediction. A more complete discussion of these models is beyond the scope of this chapter.

Finally, specifying the working etiologic (or prediction) model requires particular attention to the missingness of the data, potential intermediates, and informative censoring to ensure the veracity of results from cohort studies. Missing data are an important source of bias, particularly when not at random, such as the case when couples leave ART treatment for specific reasons [29]. Other examples of important sources of missingness include our inability to know the precise timing when couples' fecundity returns and when they become at risk for pregnancy (e.g., TTP), or our inability to identify the precise timing of embryonic or fetal death.

Intermediates are events in the pathway between exposures and outcomes, such as birth weight when studying maternal cigarette smoking and infant outcomes. Often, intermediates are controlled either in the design stage by restriction or in the analytic phase by stratification or modeling techniques. However, often such adjustment is made without any indication or without simultaneous control of the common sources for both the intermediate and outcome. Such inappropriate adjustment may introduce bias or diminish precision leading to incorrect or paradoxical results [41, 42].

Censoring arises from the loss of study participants, which is a particular concern for prospective cohort designs. As a result, the exact timing of the outcome is unobserved. Censoring is further

defined to include left, right, or interval censoring. Left censoring denotes that the outcome occurred before the study's follow-up interval. Left censoring is typically a consideration in pregnancy cohorts, in that conception occurred before enrollment, but its precise timing is unknown. Right censoring denotes that the outcome occurred after the study's follow-up interval. Right censoring is a concern with many cohort studies including TTP or ART follow-up cohorts when pregnancy occurs either after the study or during a rest cycle, respectively. Interval censoring is a unique feature of reproductive health, in that an outcome is known to occur but only within an interval. Embryonic or fetal demise is an example of such censoring, in that the exact timing of death is often unknown. Close monitoring of cohorts is needed to ensure any censoring including attrition rates are unrelated to the study outcome.

Truncation is a closely related methodologic consideration to censoring and refers to the window of observation and not study participants, per se. Left truncation needs to be accounted for in many fecundity studies when another outcome occurs before the study's follow-up. For example, couples discontinuing contraception for purposes of becoming pregnant may experience an occult pregnancy loss before enrollment into a TTP cohort study.

Despite these as well as many other methodologic considerations impacting the design of cohort studies, there are many available analytic techniques available to empirically assess their impact on study findings. Still, there is no substitution for valid and reliable data collection, including measurement of time-varying exposures and utilization of statistical methods that account for these dynamic variables that are the hallmark of prospective cohort designs focusing on reproductive health.

20.3 Cohort Study Design

20.3.1 What Is a Cohort Design?

A cohort design is a design in which a well-defined group of individuals are followed to identify new or incident disease or a health outcome. There are various ways to define the cohort, including the presence/absence of a particular exposure (e.g., multivitamin use), behavior (e.g., discontinuing contraception to become pregnant/commencing ART), or unique group membership (e.g., health maintenance organization). The duration of follow-up can be relatively short (e.g., ART or menstrual cycle), represent a critical or sensitive window (e.g., pregnancy) or last decades (e.g., birth cohorts followed until adulthood or beyond). At specified intervals or at the completion of follow-up, cohort members are compared by their exposure status in relation to study outcomes. When the exposure, behavior, or defining event is not randomly assigned to cohort members, it is an observational rather than an experimental design. When the exposure is assigned, it is an interventional or randomized controlled trial design. The fundamental characteristics of a traditional cohort design include (1) the cohort is free from disease at enrollment; (2) exposure status is defined at enrollment and sometimes again during follow-up; (3) the study's outcome(s) is(are) determined for all cohort members; and (4) data on known or possible confounders or covariates are measured at baseline or before disease occurrence, given the nonexperimental nature of the design.

Investigators have the option depending upon the research question to establish one cohort with a heterogeneous range of exposure among its members or to establish separate cohorts on the basis of exposure status. In the latter case, one cohort is presumed exposed or to possess the unique characteristic, while the other cohort is unexposed or lacking the unique characteristic. In addition, an exposure cohort can be matched to an unexposed cohort on factors that may impact disease occurrence. This approach is called a matched exposure cohort design [43].

20.3.2 Are There Subtypes of Cohort Designs?

The cohort design is actually flexible, in that it can be further characterized as being either prospective or retrospective in nature. These qualifiers pertain to the timing of when exposure

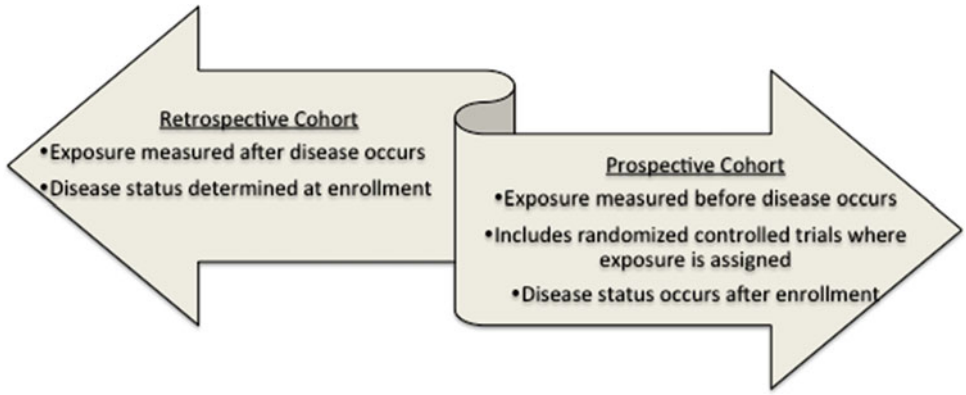

Fig. 20.3 Illustration of retrospective and prospective cohort design studies

and disease or the study outcome occurs relative to the timing of exposure data collection. To this end, if cohort identification and data collection occurs at measurement of the exposure with follow-up to identify outcomes, it is a prospective cohort. If exposure or both exposure and outcomes have occurred prior to cohort identification, and therefore data are collected from and limited to existing information, it is a retrospective cohort. Figure 20.3 illustrates these subtle but important differences regarding the timing of exposure and disease status. The prospective cohort design is, perhaps, most commonly known and utilized in clinical and epidemiological research and is the only design that can incorporate intervention/randomized controlled trial. Its great advantage is the ability to collect the exposure and potential confounding or effect modifying factor data in the breadth and depth desired (when biologically or technologically feasible) and also while allowing for variation in these factors across time as the realities of the participant's experiences unfold in real time.

Still, the retrospective cohort design can be particularly powerful and cost-effective in situations where good historical exposure data can be retrospectively ascertained. Many environmental or occupational retrospective cohorts have been successfully conducted. An example of a more recent historic cohort would be the enrollment of couples completing ART, where the spectrum of outcomes (e.g., oocyte stimulation and retrieval through embryo evaluation and transfer and ultimately implantation through delivery) is known requiring retrospective ascertainment of various exposures or lifestyle factors that occurred prior to the outcome(s) from partners or the couple.

Studies of TTP are another example of when a prospective and retrospective cohort study can be considered. The gold standard remains a prospective cohort design with preconception recruitment and enrollment of couples discontinuing contraception who are then queried on exposures and followed prospectively for a specified period of time such as 12 menstrual cycles [8]. Still, many retrospective TTP cohort studies have been conducted. In such instances, a cohort of pregnant women is recruited and established and then are followed through delivery (often with their offspring being prospectively followed) with retrospective ascertainment of exposures including TTP [44]. However, bidirectional reporting errors in TTP have been found when comparing retrospective to prospective TTP, when assuming prospective to be the gold standard [45].

More recently, a number of hybrid cohort studies have been developed with varying degrees of utilization by researchers including those interested in reproductive health. These include the case–cohort and the case–crossover designs. Each of these designs, however, has a number of important methodologic considerations that are beyond the scope of this chapter. The reader is encouraged to consult one of many good methodologic textbooks on cohort and hybrid designs to ensure the designs are properly implemented. A brief description follows; however, more complete details of each as

they pertain to reproductive health is presented elsewhere [46].

The case–cohort design was proposed by Prentice and Pyke (1979) and is useful for analyzing event time such as disease occurrence in a cohort [47]. This design compares all participants having the outcome under study with a random sample of the overall cohort established at baseline before disease or the study outcome occurs. The unique aspect of this design is that multiple "case" groups can be selected and compared with one random comparison group selected from the overall cohort. Given the many outcomes that can be assessed in a cohort study, this design is well suited for implementation. ART research, for example, lends itself to investigation of a spectrum of treatment outcomes all of which are clinically relevant and informative. Unlike a nested case–control study that assesses all cases and all controls, only a random sample of participants needs to be selected for the subcohort.

The case–crossover design allows individuals to serve as their own case and control as they are prospectively followed [48]. Given that each person contributes his/her comparison information, no external comparison group is needed. In this design, study participants experiencing an acute outcome are queried about exposures preceding the event along with other time periods that serve as a comparison interval. While this design originated with cardiovascular epidemiology in looking at myocardial infarction, it is appropriate for "acute" outcomes such as conception. Such a design would allow researchers to determine what is different about the menstrual cycle women became pregnant relative to previous nonpregnant cycles. Several strong assumptions underlie this design including no systematic changes in the exposure or relevant covariates over the study time period.

20.3.3 What Kind of Exposures Can Be Studied in a Cohort Design?

Much of this answer depends upon the research question under study. Some exposures may be fixed in that they do not change from enrollment or baseline (e.g., age at menarche in a cohort of adult women). More typically, perhaps, are exposures that have the ability to change, such as couples' lifestyles that include dietary or other behavioral (e.g., exercise and alcohol use) types of exposure that are subject to change. Also, change can be in either direction and can be motivated by pregnancy intentions or becoming pregnant.

One of the unique aspects about a cohort design is its ability to assess a range of exposures, assuming all are measured at baseline or some time interval prior to the onset of disease. As noted earlier, a hierarchical data structure is typical of most cohort studies focusing on reproductive health and will include a multitude of exposures or relevant covariates that need to be measured. Exposure status, however defined, is ascertained at baseline typically defined as upon enrollment, and it may represent a host of environmental agents, lifestyle, or behaviors or it can characterize a particular characteristic that is a determinant of the study's outcome.

Timing of exposures is an important aspect that requires careful planning for both retrospective and prospective cohort studies. Exposures must temporally fall before the outcome. Measurement of exposures is dependent upon the a priori defined unit of analysis such as couple, woman, cycle, or day level. Cohort studies often have time-varying covariates reflecting important time intervals, and the measurement of time is dependent upon the unit of analysis. For example, when analyzing ART cycles, the unit may be the treatment cycle, which cannot be exchanged with calendar time in the analysis. Similarly, if female or male partners are the unit of analysis, they cannot be exchanged for couples or vice versa. Changes in the time scale during the analysis phase of research may result in a loss of statistical power or the emergence of time/secular trend/age-related confounding that biases study results.

20.3.4 What Kind of Endpoints Can Be Studied in Cohort Designs?

As illustrated in Fig. 20.1a, b, cohort studies are well suited to the simultaneous evaluation of a spectrum of outcomes relative to a particular exposure or set of exposures. For example, a

prospective TTP cohort study can assess exposures in relation to semen quality, menstruation, ovulation, pregnancy, and infertility. If this same cohort is followed through pregnancy, it can assess gravid health or disease, gestation, and infant outcomes as recently demonstrated [26]. Cohort studies can be the platform for transgenerational research, as has been done for some of the participating clinical sites in the U.S. Collaborative Perinatal Project [49, 50]. What is particularly needed is the continued follow-up of established cohorts to assess fecundity and fertility and its implications across the lifespan. Examples of such novel research include the Nurses' Health Studies I and II [51, 52], although due to age at cohort enrollment (30–55 and 25–42 in 1976 or in 1989, respectively) pregnancy history was self-reported rather than prospectively observed for a large portion of participants. The Growing Up Today Study (GUTS), a cohort comprised of 27,000 children of the Nurses' Health Study II participants who are now in the 20s has been collecting data since these young adults were prepubescent, affording true prospective cohort evaluation of reproductive health as well as intergenerational associations [53].

20.3.5 What Are Special Considerations to Keep in Mind About a Cohort Study?

Despite its utility for reproductive health and its many strengths, noninterventional cohort studies remain observational designs and are subject to bias. Exposures are not randomly assigned in observational cohort designs precluding its ability to directly assess causality. If well designed and implemented, cohort designs are powerful tools for understanding associations between a range of exposures, behaviors, or events and a range of study outcomes. Still, this design is particularly sensitive to attrition in that perfect follow-up of the study cohort is the exception rather than the rule. Without complete follow-up of the cohort, it may not be possible to directly estimate the relative risk (RR), which is defined as the risk of disease or the study outcome in the exposed versus the unexposed. As a result, the odds ratio (OR) is estimated as a measure of association between the exposure and study outcome, or the odds of exposure among individuals with disease or the study outcome to the odds of exposure among individuals without the disease or study outcome. A second key consideration with the cohort design is competing risk, in that study participants may develop another disease or outcome under study. Competing risk can impact the interpretation of results.

Cohort inclusion criteria and thus generalizability are critical considerations as well, as evidenced by the discoveries in investigations of the relation between exogenous hormone replacement therapy and risk of coronary heart disease. Specifically, differences between protective effects observed within observational prospective cohort studies and prospective randomized controlled trials have lead to advancement of the field of cardiology, with respect to the effect of estrogens and progestins on women who are peri- or recently menopausal versus those who are postmenopausal for 5 or more years [54, 55]. As stated above, this concept of population inclusion influences the interpretation of observations within populations of pregnancy planners compared to those who conceive without intention as well as evidence gleaned from infertile populations undergoing ART compared to populations with unproven fertility or the infertile who do not seek or receive ART.

The cohort design has considerable utility for reproductive health, given the narrow time intervals for many of its outcomes and the interrelatedness and conditional nature of reproduction that results in a spectrum of relevant outcomes. As with any investigation, the research question will define the type of study design best suited for obtaining answers to critical data gaps along with other practical considerations such as the availability of target populations for sampling and establishing cohorts and available resources.

20.4 Relevancy of Reproductive Health Across the Lifespan

Reproductive health is emerging as an important marker of both the early origins of health and disease and regarding later onset disease. The early

origin of health and disease hypothesis posits [56] that many diseases arise shortly following conception, if not during the periconception period. A classic example of this hypothesis is diethylstilbestrol (DES), where gestational-specific exposures are associated with a spectrum of outcomes including cancer, structural malformations involving reproductive organs and fecundity in both male and female offspring [57–60]. Other examples include exposure to in utero androgens during sensitive windows and polycystic ovarian syndrome in nonhuman primates [61], or evidence that women with endometriosis may be smaller at birth [62] with leaner trajectories through time of diagnosis [63, 64]. Similarly, fathers of sons with hypospadias are reported to have poorer semen quality than fathers of unaffected sons [65].

Of late, two evolving paradigms suggest that human fecundity and fertility not only have an early origin but are also highly informative for health and disease across the lifespan. The first such paradigm is the testicular dysgenesis hypothesis (TDS), which was developed by Skakkebaek and colleagues [66]. The TDS hypothesis postulates that genital-urinary malformations, poor semen quality, and testes cancer may have a shared in utero etiology [67]. Such early exposures may in fact have transgenerational effects. The TDS hypothesis influenced development of the ovarian dysgenesis syndrome (ODS) paradigm [68]. Similar to men, women's fecundity may arise in utero with related impairments such as PCOS and endometriosis arising during early reproductive ages, which in turn impact gravid health and later onset adult health. For example, women with PCOS are at increased risk for gestational diabetes and metabolic and cardiovascular disease in later years [69, 70]. While no association has been established between endometriosis and gravid disease, affected women may be at increased risk for developing autoimmune disorders and cancers in comparison to women without endometriosis [71–73]. Thus, the origin of reproductive health may arise early and have implications for health across the lifespan beyond fecundity and fertility endpoints.

20.5 Conclusions

In summary, the cohort design, whether retrospective or prospective, is a powerful tool in the search for the determinants and consequences of reproductive health across the life course. Temporality, time-varying exposure and covariate data, hierarchical clustering, and correlated outcomes can be finely defined and quantified whether in population-based studies giving rise to time-to-pregnancy or life course investigation or in hospital-based studies of ART allowing a window into gamete, embryologic, and uterine biology that are otherwise unobservable. The depth and breadth and intricacy of questions remaining to be answered, and cohort designs are an invaluable tool of discovery.

References

1. Sadi N (1994) The right to reproductive and sexual health. http://www.un.org/ecosocdev/geninfo/women/womrepro.htm
2. Buck Louis G, Platt R. Introduction to reproductive and perinatal epidemiology. In: Buck Louis G, Platt R, editors. Reproductive and perinatal epidemiology. New York: Oxford University Press; 2011. p. 3–15.
3. Bonde JP, Hjollund NH, Jensen TK, et al. A follow-up study of environmental and biologic determinants of fertility among 430 Danish first-pregnancy planners: design and methods. Reprod Toxicol. 1998;12(1):19–27.
4. Sharpe RM. Environmental/lifestyle effects on spermatogenesis. Philos Trans R Soc Lond B Biol Sci. 2010;365(1546):1697–712. doi:10.1098/rstb.2009.0206.
5. Bongaarts J. A method for the estimation of fecundability. Demography. 1975;12(4):645–60.
6. Roberts C, Lowe C. Where have all the conceptions gone? Lancet. 1975;305:498–9.
7. Macklon NS, Geraedts JP, Fauser BC. Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. Hum Reprod Update. 2002;8(4):333–43.
8. Finer LB, Kost K. Unintended pregnancy rates at the state level. Perspect Sex Reprod Health. 2011;43(2):78–87. doi:10.1363/4307811.
9. Miller AB, Barclay TH, Choi NW, et al. A study of cancer, parity and age at first pregnancy. J Chronic Dis. 1980;33(10):595–605.
10. Whittaker PG, Taylor A, Lind T. Unsuspected pregnancy loss in healthy women. Lancet. 1983;1(8334):1126–7.

11. Sweeney AM, Meyer MR, Mills JL, et al. Evaluation of recruitment strategies for prospective studies of spontaneous abortion. J Occup Med. 1989;31(12):980–5.
12. France JT, Graham FM, Gosling L, et al. A prospective study of the preselection of the sex of offspring by timing intercourse relative to ovulation. Fertil Steril. 1984;41(6):894–900.
13. Ellish NJ, Saboda K, O'Connor J, et al. A prospective study of early pregnancy loss. Hum Reprod. 1996;11 (2):406–12.
14. de Mouzon J, Spira A, Schwartz D. A prospective study of the relation between smoking and fertility. Int J Epidemiol. 1988;17(2):378–84.
15. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. N Engl J Med. 1988;319(4): 189–94. doi:10.1056/NEJM198807283190401.
16. Vartiainen H, Suonio S, Halonen P, et al. Psychosocial factors, female fertility and pregnancy: a prospective study–part II: pregnancy. J Psychosom Obstet Gynaecol. 1994;15(2):77–84.
17. Hakim RB, Gray RH, Zacur H. Infertility and early pregnancy loss. Am J Obstet Gynecol. 1995;172(5): 1510–7.
18. Zinaman MJ, Clegg ED, Brown CC, et al. Estimates of human fertility and pregnancy loss. Fertil Steril. 1996;65(3):503–9.
19. Columbo B, Masarotto G. Daily fecundability: first results from a new database. Demogr Res. 2000;3(5).
20. Wang X, Chen C, Wang L, et al. Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. Fertil Steril. 2003;79(3):577–84.
21. Pyper C, Amery J, Watson M, et al. Patients' experiences when accessing their online electronic patient records in primary care. Br J Gen Pract. 2004;54(498): 38–43.
22. Mikkelsen EM, Hatch EE, Wise LA, et al. Cohort profile: the Danish Web-based Pregnancy Planning Study–'Snart-Gravid'. Int J Epidemiol. 2009;38(4): 938–43. doi:10.1093/ije/dyn191.
23. Brown SA, Collins PW. Oral anticoagulants and fetal loss. Lancet. 1996;348(9043):1734.
24. Buck GM, Tee PG, Fitzgerald EF, et al. Maternal sport fish consumption and infant birth size and gestation: New York State Angler Cohort Sudy. Environ Health. 2002;2(7):1–9.
25. Buck Louis GM, Lum KJ, Sundaram R, et al. Stress reduces conception probabilities across the fertile window: evidence in support of relaxation. Fertil Steril. 2011;95(7):2184–9. doi:10.1016/j.fertnstert.2010.06.078.
26. Buck Louis GM, Schisterman EF, Sweeney AM, et al. Designing prospective cohort studies for assessing reproductive and developmental toxicity during sensitive windows of human reproduction and development–the LIFE Study. Paediatr Perinat Epidemiol. 2011;25(5):413–24. doi:10.1111/j.1365-3016.2011.01205.x.
27. Slama R, Ducot B, Carstensen L, et al. Feasibility of the current-duration approach to studying human fecundity. Epidemiology. 2006;17(4):440–9. doi:10.1097/01.ede.0000221781.15114.88.
28. Missmer SA, Pearson KR, Ryan LM, et al. Analysis of multiple-cycle data from couples undergoing in vitro fertilization: methodologic issues and statistical approaches. Epidemiology. 2011;22(4):497–504. doi:10.1097/EDE.0b013e31821b5351.
29. Pearson KR, Hauser R, Cramer DW, et al. Point of failure as a predictor of in vitro fertilization treatment discontinuation. Fertil Steril. 2009;91(4 Suppl):1483–5. doi:10.1016/j.fertnstert.2008.07.1732.
30. Buck Louis GM, Dukic VM, Heagerty PJ, et al. Statistical issues in modeling pregnancy outcome data. Stat Methods Med Res. 2006;15(2):103–26.
31. Buck Louis GM, Schisterman EF, Dukic VM, et al. Research hurdles complicating the analysis of infertility treatment and child health. Hum Reprod. 2005;20(1):12–8. doi:10.1093/humrep/deh542.
32. Howards PP, Schisterman EF, Heagerty PJ. Potential confounding by exposure history and prior outcomes: an example from perinatal epidemiology. Epidemiology. 2007;18(5):544–51.
33. Therneau TM, Grambsch PM. Modeling survival data: extending the Cox Model, Springer Series. New York: Statistics for Biology and Health; 2000.
34. Paneth N. Invited commentary: the hidden population in perinatal epidemiology. Am J Epidemiol. 2008;167(7):793–6. doi:10.1093/aje/kwn041. author reply 797–798.
35. McLain AC, Sundaram R, Cooney MA, et al. Clustering of fecundability within women. Paediatr Perinat Epidemiol. 2011;25(5):460–5. doi:10.1111/j.1365-3016.2011.01219.x.
36. Basso O, Olsen J, Bisanti L, et al. Repeating episodes of low fecundability. A multicentre European study. The European Study Group on Infertility and Subfecundity. Hum Reprod. 1997;12(7):1448–53.
37. Ananth CV. Epidemiologic approaches for studying recurrent pregnancy outcomes: challenges and implications for research. Semin Perinatol. 2007;31(3):196–201. doi:10.1053/j.semperi.2007.03.008.
38. Dukic V, Hogan JW. A hierarchical Bayesian approach to modeling embryo implantation following in vitro fertilization. Biostatistics. 2002;3(3):361–77. doi:10.1093/biostatistics/3.3.361.
39. Daya S. Pitfalls in the design and analysis of efficacy trials in subfertility. Hum Reprod. 2003;18(5): 1005–9.
40. Hogan JW, Blazar AS. Hierarchical logistic regression models for clustered binary outcomes in studies of IVF-ET. Fertil Steril. 2000;73(3):575–81.
41. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology. 2009;20(4):488–95. doi:10.1097/EDE.0b013e3181a819a1.
42. VanderWeele TJ, Mumford SL, Schisterman EF. Conditioning on intermediates in perinatal epidemiology. Epidemiology. 2012;23(1):1–9. doi:10.1097/EDE.0b013e31823aca5d.
43. Rothman KJ, Greenland S, Lash TL (2008) Cohort studies. In: Modern epidemiology. 3rd ed. Philadelphia: Lippincott Williams and Wilkins. p. 100–10.

44. Schisterman EF. Novel study designs and their application in reproductive and perinatal epidemiology. In: Buck Louis GM, Platt RW, editors. Reproductive and perinatal epidemiology. New York: Oxford University Press; 2011. p. 314–28.
45. Cooney MA, Buck Louis GM, Sundaram R, et al. Validity of self-reported time to pregnancy. Epidemiology. 2009;20(1):56–9. doi:10.1097/EDE.0b013e31818ef47e.
46. Basso O, Olsen J. Subfecundity and neonatal mortality: longitudinal study within the Danish national birth cohort. BMJ. 2005;330(7488):393–4. doi:10.1136/bmj.38336.616806.8F.
47. Prentice RL, Pike R. Logistic disease incidence models and case-control studies. Biometrika. 1979;66(3):403–11.
48. Maclure M. The case-crossover design: a method for studying transient effects on the risk of acute events. Am J Epidemiol. 1991;133(2):144–53.
49. Wen X, Shenassa ED, Paradis AD. Maternal smoking, breastfeeding, and risk of childhood overweight: findings from a national cohort. Matern Child Health. 2012;17(4):746–55.
50. Wasserman EE, Pillion JP, Duggan A, et al. Childhood IQ, hearing loss, and maternal thyroid autoimmunity in the Baltimore Collaborative Perinatal Project. Pediatr Res. 2012;72(5):525–30. doi:10.1038/pr.2012.117.
51. Rich-Edwards JW, Goldman MB, Willett WC, et al. Adolescent body mass index and infertility caused by ovulatory disorder. Am J Obstet Gynecol. 1994;171(1):171–7.
52. Lipnick RJ, Buring JE, Hennekens CH, et al. Oral contraceptives and breast cancer. A prospective cohort study. JAMA. 1986;255(1):58–61.
53. Gillman MW, Rifas-Shiman SL, Camargo Jr CA, et al. Risk of overweight among adolescents who were breastfed as infants. JAMA. 2001;285(19):2461–7.
54. Manson JE, Bassuk SS, Harman SM, et al. Postmenopausal hormone therapy: new questions and the case for new clinical trials. Menopause. 2006;13(1):139–47. doi:10.1097/01.gme.0000177906.94515.ff.
55. Willett WC, Manson JE, Grodstein F, et al. Re: "combined postmenopausal hormone therapy and cardiovascular disease: toward resolving the discrepancy between observational studies and the women's health initiative clinical trial". Am J Epidemiol. 2006;163(ll):1067–8. doi:10.1093/aje/kwj156. author reply 1068–1069.
56. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. Science. 2004;305(5691):1733–6. doi:10.1126/science.1095292.
57. Hoover RN, Hyer M, Pfeiffer RM, et al. Adverse health outcomes in women exposed in utero to diethylstilbestrol. N Engl J Med. 2011;365(14):1304–14. doi:10.1056/NEJMoa1013961.
58. Kaufman RH. Structural changes of the genital tract associated with in utero exposure to diethylstilbestrol. Obstet Gynecol Annu. 1982;11:187–202.
59. Palmer JR, Hatch EE, Rao RS, et al. Infertility among women exposed prenatally to diethylstilbestrol. Am J Epidemiol. 2001;154(4):316–21.
60. Brouwers MM, Feitz WF, Roelofs LA, et al. Hypospadias: a transgenerational effect of diethylstilbestrol? Hum Reprod. 2006;21(3):666–9. doi:10.1093/humrep/dei398.
61. Abbott DH, Dumesic DA, Franks S. Developmental origin of polycystic ovary syndrome – a hypothesis. J Endocrinol. 2002;174(1):1–5.
62. Missmer SA, Hankinson SE, Spiegelman D, et al. In utero exposures and the incidence of endometriosis. Fertil Steril. 2004;82(6):1501–8. doi:10.1016/j.fertnstert.2004.04.065.
63. Hediger ML, Hartnett HJ, Louis GM. Association of endometriosis with body size and figure. Fertil Steril. 2005;84(5):1366–74. doi:10.1016/j.fertnstert.2005. 05.029.
64. Vitonis AF, Baer HJ, Hankinson SE, et al. A prospective study of body size during childhood and early adulthood and the incidence of endometriosis. Hum Reprod. 2010;25(5):1325–34. doi:10.1093/humrep/deq039.
65. Asklund C, Jorgensen N, Skakkebaek NE, et al. Increased frequency of reproductive health problems among fathers of boys with hypospadias. Hum Reprod. 2007;22(10):2639–46. doi:10.1093/humrep/dem217.
66. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001;16(5):972–8.
67. Moller H. Trends in sex-ratio, testicular cancer and male reproductive hazards: are they connected? APMIS. 1998;106(1):232–8. discussion 238–239.
68. Buck Louis GM, Cooney MA, Peterson CM. The ovarian dysgenesis syndrome. J Dev Origin Health Dis. 2011;2(1):25–35.
69. Fauser BC, Tarlatzis BC, Rebar RW, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril. 2012;97(1):28–38.e25. doi:10.1016/j.fertnstert.2011.09.024.
70. Boomsma CM, Eijkemans MJ, Hughes EG, et al. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. Hum Reprod Update. 2006;12(6):673–83. doi:10.1093/humupd/dm1036.
71. Sinaii N, Cleary SD, Ballweg ML, et al. High rates of autoimmune and endocrine disorders, fibromyalgia, chronic fatigue syndrome and atopic diseases among women with endometriosis: a survey analysis. Hum Reprod. 2002;17(10):2715–24.
72. Brinton LA, Gridley G, Persson I, et al. Cancer risk after a hospital discharge diagnosis of endometriosis. Am J Obstet Gynecol. 1997;176(3):572–9.
73. Brinton LA, Sakoda LC, Sherman ME, et al. Relationship of benign gynecologic diseases to subsequent risk of ovarian and uterine tumors. Cancer Epidemiol Biomarkers Prev. 2005;14(12):2929–35. doi:10.1158/1055-9965.EPI-05-0394.

Index

P.N. Schlegel et al. (eds.), *Biennial Review of Infertility: Volume 3*,
DOI 10.1007/978-1-4614-7187-5, © Springer Science+Business Media New York 2013