Pak H. Chung
Zev Rosenwaks   *Editors*

Problem-Focused Reproductive Endocrinology and Infertility

Springer

Contemporary Endocrinology

Series Editor
Leonid Poretsky, Division of Endocrinology
Lenox Hill Hospital
New York, NY, USA

Contemporary Endocrinology offers an array of titles covering clinical as well as bench research topics of interest to practicing endocrinologists and researchers. Topics include obesity management, androgen excess disorders, stem cells in endocrinology, evidence-based endocrinology, diabetes, genomics and endocrinology, as well as others. Series Editor Leonid Poretsky, MD, is Chief of the Division of Endocrinology and Associate Chairman for Research at Lenox Hill Hospital, and Professor of Medicine at Hofstra North Shore-LIJ School of Medicine.

Pak H. Chung • Zev Rosenwaks
Editors

Problem-Focused Reproductive Endocrinology and Infertility

🐎 Springer

Editors
Pak H. Chung
Ronald O. Perelman and Claudia Cohen
Center for Reproductive Medicine
Weill Medical College of Cornell University
New York, NY, USA

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

Series Editor Foreword

Truly amazing advances occur in reproductive medicine with immense speed. It is therefore becoming extremely important to provide factual up-to-date information on reproductive issues to health professionals both for their daily work and for their efforts in educating the general public.

In this regard, the volume edited by Drs. Pak Chung and Zev Rosenwaks presents an invaluable tool. Written by a stellar interdisciplinary faculty crew associated with a world-renowned Center for Reproductive Medicine at Weill Cornell Medical College, this text covers completely and succinctly the entire field of reproductive medicine.

The case-based approach chosen by the authors and editors makes the book particularly useful for clinicians as well as students of reproductive medicine at all levels of training. Publication of this comprehensive volume, excellent in both content and form, could not be more timely. Congratulations are in order to the editors and all authors for their impressive accomplishment.

Leonid Poretsky
Division of Endocrinology
Gerald J. Friedman Diabetes Institute
The Gerald J. Friedman Transgender Program for Health and Wellness
Department of Medicine, Lenox Hill Hospital
New York, NY, USA

Hofstra Northwell School of Medicine
and Feinstein Institutes for Medical Research
Manhasset, NY, USA

Preface

We are eminently pleased and excited to present this handbook, titled *Problem-Focused Reproductive Endocrinology and Infertility*. It is our hope that this foundational contribution will become a useful resource for students, residents, fellows, reproductive endocrinologists, and other medical professionals who provide care for infertility and reproductive disorders. This comprehensive compendium is one of a series of more than 80 titles published by Springer, Contemporary Endocrinology, that address a variety of clinical and research topics in endocrinology.

This book offers two special features. First, all the authors are faculty members in the Departments of Reproductive Medicine, Urology, and Internal Medicine at Weill-Cornell Medicine. Second, the titles of the chapters are problem- or focus-based. Each is either a chief complaint or a problem encountered in clinical practice. Every chapter is launched with an authentic case presentation followed by an in-depth discussion of the topic. The authors strive to provide the physiological and pathophysiological basis for each clinical problem along with the available evidence-based contemporary therapy.

The collective contributions of our Weill Cornell colleagues not only represent a distillation of many years of clinical experience but also provide a glimpse into their individual and often unique clinical approaches. We anticipate that our readers will find the material useful and impactful in their clinical practice.

Our special thanks to all the contributors who made this project possible.

New York, NY, USA   Pak H. Chung
New York, NY, USA   Zev Rosenwaks

Contents

Contributors

Ashley Aluko, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Pietro Bortoletto, M.D., B.Sc. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Stephanie Cheung, M.Sc., ALS (ABB) Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY, USA

Pak H. Chung, M.D. Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Owen Davis, M.D. Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY, USA

Rony Elias, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Dan Goldschlag, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Marc Goldstein, M.D. Male Reproductive Medicine and Surgery, Weill Medical College of Cornell University, New York, NY, USA

Elizabeth Grill, Psy.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Kolbe Hancock, M.D. Reproductive Medicine and Ob/Gyn, Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Caroline Kang, M.D. Urology, Department of Urology, Weill Medical College of Cornell University, New York, NY, USA

Hey-Joo Kang, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

James Kashanian, M.D. Urology and Reproductive Medicine, Male Sexual Health, Department of Urology, Weill Medical College of Cornell University, New York, NY, USA

Melissa D. Katz, M.D. Department of Medicine, Division of Endocrinology, Weill Medical College of Cornell University, New York, NY, USA

Derek Keating, B.A. Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY, USA

Isaac Kligman, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Alexis Melnick, M.D. Reproductive Medicine and Ob/Gyn, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

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

Alessandra Parrella, M.Sc. Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY, USA

Nigel Pereira, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Allison Petrini, M.D. Reproductive Medicine and Ob/Gyn, Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Samantha M. Pfeifer, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Meridith Pollie, B.Sc. Weill Medical College of Cornell University, New York, NY, USA

Nahid Punjani, M.D. Department of Urology, Weill Medical College of Cornell University, New York, NY, USA

David Reichman, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Phillip Romanski, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

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

Glenn Schattman, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Peter Schlegel, M.D. Department of Urology, Weill Medical College of Cornell University, New York, NY, USA

Steven Spandorfer, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Joshua Stewart, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Nina Vyas, M.D. Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

Philip Xie, B.Sc. Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY, USA

Chapter 1
Ambiguous Genitalia

Meridith Pollie and Samantha M. Pfeifer

Case

A 3630-g infant was delivered vaginally 1 h ago after an uncomplicated 39-week gestation. On examination, the external genitalia are ambiguous. Vital signs are stable and the infant does not appear to be in acute distress. Examination shows small scrotal sacs resembling enlarged labia without palpable testes. An enlarged clitoris vs microphallus with hypospadias is seen. There is a small vaginal opening that appears to be partially fused. The remainder of the examination is normal.

Differential Diagnosis

The presenting symptom in this infant is ambiguous genitalia, or genitalia that do not appear typically male or female. The differential diagnosis of ambiguous genitalia in a newborn baby includes disorders of sexual development (DSD), in utero exposure to hormones that may masculinize female genitalia, insufficient androgen exposure in a male fetus, substances such as phenytoin and phenobarbital that may feminize male genitalia, and conditions of maternal hyperandrogenism such as maternal luteomas or theca lutein cysts that may also masculinize female genitalia. A comprehensive history should include hormone, drug, or substance exposure.

A disorder of sexual development is the most likely etiology in this infant. DSD are characterized by a mismatch between chromosomal/gonadal sex and genital

M. Pollie
Weill Medical College of Cornell University, New York, NY, USA

S. M. Pfeifer (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: spfeifer@med.cornell.edu

development, and etiologies include: genetic causes, referring to the presence or absence of functional sex chromosomes; signaling causes, referring to disrupted cellular communication during embryonic development; hormonal causes, referring to altered production or function of enzymes involved in hormone synthesis; and hormone receptor defects, which result in unsuccessful receptor-ligand interactions. Because developmental pathways are largely determined by an individual's karyotype, the latter is the cornerstone in guiding diagnosis of these patients.

For patients with XX karyotype, ambiguous genitalia result from pathologies involving high levels of androgens, either from the maternal environment or increased production in the fetal gonads, adrenal glands, or ectopic tissue. Congenital adrenal hyperplasia (CAH) includes a group of syndromes resulting from a deficiency in one of the enzymes needed to synthesize aldosterone and cortisol, leading to overproduction of androgens. More than 90% of CAH cases are caused by 21-hydroxylase deficiency, which leads to elevated serum levels of 17-hydroxyprogesterone (17-OHP) and androgens [1]. The most severe form of classic 21-hydroxylase deficiency can result in a salt-wasting syndrome due to dangerously low levels of serum aldosterone, leading to hyponatremia, hyperkalemia, metabolic acidosis, and inappropriate urine sodium excretion [1]. Infants with the salt-wasting syndrome are at risk for hypovolemic and hypoglycemic adrenal crises which can be fatal [1]. Other serum hormone levels, including cortisol, dehydroepiandrosterone, 17-hydroxypregnenolone, and 11-deoxycortisol, can help differentiate 21-hydroxylase deficiency from other, less common subtypes of CAH, including 3-beta-hydroxysteroid dehydrogenase deficiency or 11-beta-hydroxylase deficiency. Placental aromatase deficiency, an important diagnosis to consider in XX newborns, can lead to both ambiguous genitalia in the infant as well as maternal virilization due to excess serum testosterone which usually resolves after delivery.

Another important group of etiologies in XX individuals are mutations or translocations in genes involved in gonadal development. These include the translocation of the *SRY* gene, necessary in driving male gonadal development, from the Y-chromosome to the X-chromosome, mutations in the *SOX9* gene, which is a necessary transcription factor for testicular development, and mutations in *NR5A1*, which can lead to gain-of-function mutations causing inappropriate testicular tissue development (in XX individual) or loss-of-function mutations causing ineffective testicular differentiation (in XY individuals).

For patients with karyotype XY, syndromes that present with ambiguous genitalia are related to abnormally low levels or activity of the male sex hormones, testosterone, and dihydrotestosterone (DHT). Disorders of androgen synthesis include Smith-Lemli-Opitz syndrome (7-dehydrocholesterol reductase deficiency), P450 oxidoreductase deficiency, and 17,20 lyase deficiency. Contrastingly, 5-alpha-reductase deficiency is characterized by normal testosterone production but abnormal conversion of testosterone into DHT, impeding the DHT-dependent virilization of external genitalia. Androgen insensitivity syndrome (AIS), on the other hand, occurs when androgen hormone production is normal, but the androgen receptor is abnormal. This syndrome encompasses a spectrum of disorders from mild to partial or complete AIS that vary in presentation based on the degree of receptor

responsiveness to androgens and therefore the degree of virilization. Finally, patients with 46,XY/45,XO mosaicism may be diagnosed with Mixed Gonadal Dysgenesis (MGD) which entails a testis with Sertoli and Leydig cells but no germinal elements on one side and a streak gonad on the other. MGD is frequently due to mutations in the *WT1*, *SRY*, or *NR5A1* genes.

Evaluation

The first step in evaluating this patient with ambiguous genitalia is to screen for disorders that could be life-threatening, notably the salt-wasting form of CAH. Approximately 75% of patients with CAH have the salt-wasting type. Infants suffer from severe mineralocorticoid deficiency which can lead to Addisonian crisis and death if steroid supplementation is not initiated immediately [1]. Therefore, it is recommended that most infants with ambiguous genitalia should be screened immediately with serum electrolytes and 17-OHP levels. A karyotype can determine the child's chromosomal make-up and identify any mosaicism, but may take days to weeks to become available. Currently, many women undergo cell-free DNA testing in the first trimester to exclude Down Syndrome and may already know the fetal karyotype. These results should be confirmed postnatally. In our patient, initial screening labs revealed normal serum electrolytes and 17-OHP, and a prenatal test confirmed XY karyotype.

Once potentially fatal disorders are ruled out, a thorough history and physical exam should be the next steps in evaluation. History-taking should include specific questions about family history of consanguinity, infertility, or gonadal/urogenital malformations, maternal history of antenatal substance or medication use and any prior pregnancies, and maternal symptoms that may suggest androgen excess [2]. Initial examination of the infant should include evaluation of hydration status, jaundice, areolar hyperpigmentation, and blood pressure abnormalities, all of which may suggest altered levels of ACTH or mineralocorticoids. A focused genital exam should evaluate the presence or absence of testicular tissue, length of the clitoris/ phallus, any fusion or rugosity of scrotal folds, hyperpigmentation, patency of the vaginal opening, and a digital rectal exam, which can reveal the presence of a uterus [2]. The external genitalia can be described using the Prader scale, which stages the degree of virilization using stages I (female with clitoromegaly) to V (male with hypospadias) [2]. Imaging with abdominopelvic ultrasound is helpful in determining the presence or absence of male/female internal genitalia. Our patient's family and maternal history was unremarkable, and examination revealed small scrotal sacs resembling enlarged labia without palpable testes, an enlarged clitoris vs microphallus with hypospadias, and a small vaginal opening that appears to be partially fused. The rectum was patent and no uterus was palpable on digital rectal exam. Abdominopelvic ultrasound revealed the absence of a uterus, upper vagina, fallopian tubes and ovaries, and the presence of bilateral intraabdominal testes.

Diagnostic workup should proceed with laboratory evaluation. In our patient, labs revealed elevated serum levels of testosterone, with normal levels of luteinizing hormone (LH), follicular stimulating hormone (FSH), and DHT. Elevated serum testosterone in a newborn is diagnostic of AIS and excludes the diagnosis of complete gonadal dysgenesis in which testosterone is not produced. Additional testing that is usually performed later in childhood includes human chorionic gonadotropin stimulation test to assess testosterone secretion by Leydig cells. Following the administration of human chorionic gonadotropin (hCG) (1000–2000 IU/day hCG for 3–5 days), an increase in testosterone (>200 ng/dL) suggests a diagnosis of AIS. Low serum testosterone after stimulation may suggest an alternative diagnosis impairing hormone synthesis, such as CAH. Normal testicular function can be further confirmed with serum anti-Mullerian hormone or inhibin, which should be within normal male limits in patients with AIS. Another useful test is to assess the ratio of serum testosterone to DHT, which should be normal in patients with AIS. An elevated testosterone/DHT ratio may suggest 5-alpha-reductase deficiency.

For patients with suspected AIS, genetic testing may offer the opportunity for definitive diagnosis. The androgen receptor (AR) gene is found on the long arm of the X chromosome and has eight exons that code for a protein of 919 amino acids [3]. Over 1000 genetic mutations causing AIS have been identified [3], initially using multiplex ligation-dependent probe amplification analysis, a type of polymerase chain reaction [4], and more recently, via next-generation and whole-exome sequencing [5]. AIS can be subclassified into complete, partial, and mild depending on the degree of responsiveness to androgens. Patients with complete AIS (CAIS) have the most severe form of androgen insensitivity and present with typical female external genitalia and testes with no internal female genitalia. CAIS has a global incidence estimated to range from 1 in 20,000 to 1 in 99,000 and is associated with identifiable mutations in more than 95% of cases [6, 7]. In CAIS patients, mutations have been identified in both exons and introns of AR gene, with the most common being missense mutations in the DNA-binding and the ligand-binding domains [3]. Mutations have also been found in CAIS patients in AR gene coactivators, helping to explain the phenotype in patients without mutations in the AR gene itself [8], as well as in the gene coding for 5-alpha-reductase [3], highlighting the overlapping features of these two disorders.

Partial AIS (PAIS) includes a spectrum of phenotypes characterized by varying degrees of masculinization of the external genitalia due to incomplete androgen responsiveness. The incidence of PAIS has been estimated to approach 1 in 130,000 [7]. Several limitations exist in genetic testing for patients with suspected PAIS. Firstly, while loss-of-function mutations in the AR gene can be found in nearly all CAIS patients, these mutations can be found in less than 50% of PAIS patients [9]. Some PAIS mutations have been found instead in the hinge region connecting DNA-binding domain and the ligand-binding domain [3]. In addition, there is no genotype-phenotype correlation in PAIS, which is to say that two individuals with the same gene mutation may have distinct clinical presentations [6]. Finally, some cases of PAIS may result from post-zygotic mutations leading to somatic mosaicism of normal wild-type receptors and abnormally mutated receptors, which

may lead to falsely normal genetic testing results [10]. In patients without identifiable mutations, androgen binding assays may provide an alternative way to quantify AR responsiveness and function.

In this section, based on karyotype, imaging, and laboratory evaluation, we have established the most likely diagnosis for our patient as PAIS.

Treatment

The next step in management of an infant determined to have PAIS should be to assemble a multidisciplinary team made up of pediatricians, endocrinologists, pediatric urologists, geneticists, and counselors who specialize in DSD and who can work together to guide the family with medical management and emotional support and guidance. Providers should acknowledge that no treatment exists to prevent or reverse abnormal development in embryogenesis in patients with PAIS. It should also be recognized that initial gender uncertainty can be distressing for families and language that pathologizes the condition should be avoided. Instead, differences in sexual development should be presented as anatomic variations. The words normal and abnormal should be replaced by typical and atypical. Case series estimate that 5–15% of PAIS children will experience gender dysphoria regardless of the sex they are raised with [11, 12]. These discussions should aim to communicate that, in infancy, it is impossible to determine the child's eventual gender identity. Therefore, parents can be encouraged to choose a gender-neutral name and prepare to rear the child with expectations that gender identity may change as the child ages. Current recommendations emphasize the importance of patient autonomy, age-appropriate education, and shared decision-making. This marks a drastic shift from previous practice of nondisclosure, an approach which stemmed from the medico-ethical climate of the 1950s that involved concealing the diagnosis of AIS from patients in an attempt to avoid confusion and minimize patient anxiety [13]. Today, ongoing psychological support for both the patient and family comprises an integral part of management and can include psychotherapy, support groups, and strong continuity with medical professionals [14].

An important question that arises in the management of patients with AIS is whether and when to pursue gonadectomy. For patients with CAIS, bilateral gonadectomy was historically recommended in childhood as cryptorchid testes were thought to carry increased risk for malignant testicular germ cell tumors [15]. However, recent literature suggests that rates of tumorigenesis in patients with CAIS may be as low as 2% [14]. Studies suggest that risk for malignant transformation may be slightly higher in patients with abdominal versus inguinal testes [16]. However, recent practice has shifted toward deferring gonadectomy until late adolescence in CAIS patients for several reasons. First, the maintenance of endogenous androgen secretion through puberty allows for bone mineral accumulation, breast development, and a growth spurt via peripheral aromatization of androgens to estrogens [15]. Second, recent evidence shows that testicular tumors do not develop until
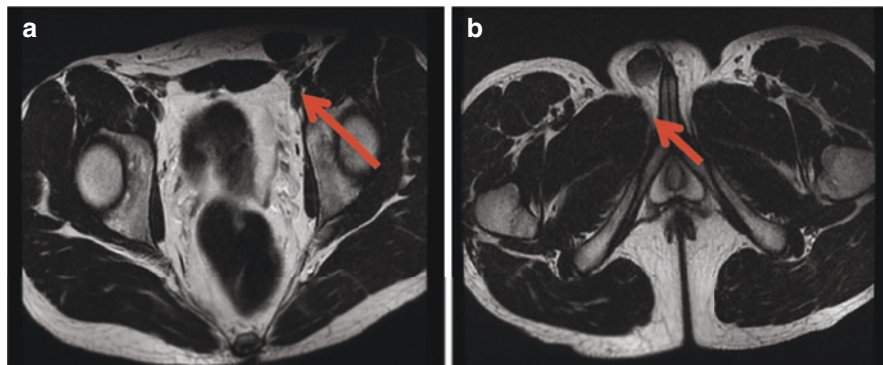
lymphadenopathy, size change, or new asymmetry), MRI should be performed, followed by possible diagnostic laparoscopy for direct visualization with possible biopsy and/or gonadectomy [7].

Patients with AIS should also be counseled about their prospects for fertility. Infertility in male-identifying patients with AIS is almost universal [20], but assisted reproductive technologies may allow for success. In CAIS testes reveal incomplete spermatogenesis, increased fibrosis, Leydig cell hyperplasia, and low frequency of spermatogonia. In PAIS individuals some androgen receptor function is preserved but not usually enough to promote adequate sperm production. Individuals with mild AIS may be diagnosed due to presence of severe oligospermia at the time of an infertility evaluation. As gonadal germ cells have been shown to be present in CAIS patients, some have proposed that the same techniques currently in use for fertility preservation in pediatric cancer patients be utilized with these patients [21]. However, as it has been shown that the number of gonadal germ cells may inversely correlate with patient age, some propose removal and cryopreservation of testicular germ cells prior to age 2 [22]. However, these procedures and timing of such are still considered experimental. For individuals with PAIS who identify as male, therapy with clomiphene citrate [23] and high-dose testosterone therapy [24] have been proposed to improve semen parameters and fertility. In patients with known heritable mutations, risk for intergenerational transmission of the disease to female offspring via the X chromosome should be discussed, and preimplantation genetic screening can be offered [21]. For female-identifying patients with AIS who desire pregnancy, options include surrogacy or potentially uterus transplant in the future, as currently individuals with a 46 XY karyotype are not candidates for this experimental procedure.

Once a patient with AIS reaches puberty, additional interventions may be indicated depending on the patient's gender identity. For AIS individuals who identify a gender other than that assigned to them at birth or in childhood, hormone therapy or surgical treatment may help to alleviate distress and affirm their desired gender expression. Hormone replacement therapy (HRT) is indicated in all individuals post-gonadectomy either at the time of expected puberty or post-puberty, depending on the timing of surgery. For those who identify as female and had a gonadectomy prior to puberty, estrogen supplementation should be initiated to induce breast development, puberty, and augment bone density. Estrogen should be initiated at the lowest dosage and gradually increased over approximately 2 years to support physiologic breast development. Individuals should then be maintained on a dosage of estrogen appropriate for a young female, which is higher than that for postmenopausal females [6]. In individuals with PAIS who identify as male, high-dose testosterone supplementation is well-documented to have a positive impact with regard to virilization [25, 26]. In individuals with CAIS who identify as female, testosterone therapy has been shown to enhance sexual function without impacting adversely on psychological well-being [27]. Some individuals with CAIS are interested in adding testosterone therapy to estrogen replacement to see if they feel "better." It is important to tailor the hormone regimen to the individual and titrate the dose to optimize well-being, secondary sexual characteristics, and bone density.

Reconstructive surgery is also an option for patients with PAIS who desire improved sexual function or the creation of more "typical"-appearing external genitalia. Though historically feminizing genitoplasty was performed in PAIS patients within the first 3 years of life [28], practice has now shifted to allow PAIS patients to delay surgery until they are old enough to make an informed decision that aligns with their chosen gender identity. Post-surgical complications are relatively uncommon but include vaginal stenosis, adhesion formation, and urethrovaginal fistulae [29]. Vaginal dilation can restore adequate vaginal depth for sexual activity in some patients. Vaginoplasty exists as an option for women who fail dilation therapy and can be performed by any available technique such as McIndoe vaginoplasty using full or split-thickness skin graft or buccal mucosa, pelvic peritoneum (Davydov procedure), or Vecchietti procedure, or bowel vaginoplasty which is more invasive [30, 31]. Success rates are dependent on post-operative management and maintenance of dilation therapy until sexually activity is regular.

Discussion

AIS can be subclassified into complete, partial, and mild depending on the physical findings which reflect the degree of responsiveness to androgens. Complete AIS, which presents with typical female external genitalia and testes with no internal female genitalia, has a global incidence estimated to range from 1 in 20,000 to 1 in 99,000. The incidence of partial AIS has been estimated to approach 1 in 130,000, though the true incidence is difficult to measure as partial AIS includes a spectrum of presentations with inconsistent correlations between clinical presentation and genetic testing results. Little is known about the incidence and prevalence of mild AIS, which presents with male external genitalia with possible impaired pubertal virilization or infertility, since many patients likely never seek care or are never diagnosed.

Today, one of the cornerstones of the treatment of patients with AIS is immediate disclosure at time of diagnosis to respect patient autonomy; however, this has not always been the case. Consensus among physicians in the 1950s was that "therapeutic privilege" should be exercised with AIS patients, meaning that doctors believed that withholding the potentially upsetting diagnosis of AIS was in the patient's best interest, as concealment would "protect" the patient from the potential psychological harm that the knowledge could bring [13]. The practice of nondisclosure remained prevalent well into the 1990s, when gynecologists were still arguing that in AIS patients, "the disclosure of genotype is irrelevant to care and may be confusing to patient and family" [32]. Fortunately, practice has shifted toward full disclosure, equipping patients with all relevant information about their medical condition so that they can make informed decisions about their ensuing care. As long as disclosure is approached in a sensitive and professional manner, patients can be empowered by the knowledge of their diagnosis rather than debilitated by it.

Patient autonomy should also guide decision-making when it comes to whether and when to pursue gonadectomy in patients with AIS. Nevertheless, all AIS patients

and their parents should be made aware of potential elevated risk for gonadal malignancy at the time of diagnosis. Risk for malignancy varies depending on AIS subtypes. Because CAIS patients have been shown to carry a malignancy risk as low as 2% and tend only to develop tumors after puberty, recent recommendations support gonadectomy in late adolescence in order to maintain endogenous hormone production through puberty for adequate growth and breast development and also facilitate including patients in decision-making [14, 15, 17, 18]. For PAIS patients, due to elevated cancer risk, particularly in those with intraabdominal testes, current recommendations are for gonadectomy at the time of diagnosis [14]. In CAIS or PAIS patients who elect to forego surgery and retain their testes, monitoring with regular screening for testicular malignancy is required. Proposed screening regimens include imaging with regular US and MRI, as well as serial tumor markers and serum hormone levels [7, 19].

References

1. Yau M, Gujral J, New MI. Congenital adrenal hyperplasia: diagnosis and emergency treatment [Internet]. MDText.com, Inc.; 2019 [cited 2022 Jan 6]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279085/.
2. Murphy C, Allen L, Jamieson MA. Ambiguous genitalia in the newborn: an overview and teaching tool. J Pediatr Adolesc Gynecol. 2011;24(5):236–50.
3. Zhang D, Yao F, Tian T, Deng S, Luo M, Tian Q. Clinical characteristics and molecular genetics of complete androgen insensitivity syndrome patients: a series study of 30 cases from a Chinese tertiary medical center. Fertil Steril. 2021;115(5):1270–9.
4. Hughes IA, Werner R, Bunch T, Hiort O. Androgen Insensitivity Syndrome. Semin Reprod Med. 2012;30(5):432–42.
5. Eggers S, Sadedin S, van den Bergen JA, Robevska G, Ohnesorg T, Hewitt J, et al. Disorders of sex development: insights from targeted gene sequencing of a large international patient cohort. Genome Biol. 2016;17(1):243.
6. Hughes IA, Davies JD, Bunch TI, Pasterski V, Mastroyannopoulou K, MacDougall J. Androgen insensitivity syndrome. Lancet. 2012;380(9851):1419–28.
7. Weidler EM, Linnaus ME, Baratz AB, Goncalves LF, Bailey S, Hernandez SJ, et al. A management protocol for gonad preservation in patients with androgen insensitivity syndrome. J Pediatr Adolesc Gynecol. 2019;32(6):605–11.
8. Adachi M, Takayanagi R, Tomura A, Imasaki K, Kato S, Goto K, et al. Androgen-insensitivity syndrome as a possible coactivator disease. N Engl J Med. 2000;343(12):856–62.
9. Batista RL, Yamaguchi K, Rodrigues A d S, Nishi MY, Goodier JL, Carvalho LR, et al. Mobile DNA in endocrinology: LINE-1 retrotransposon causing partial androgen insensitivity syndrome. J Clin Endocrinol Metab. 2019;104(12):6385–90.
10. Holterhus PM, Wiebel J, Sinnecker GH, Brüggenwirth HT, Sippell WG, Brinkmann AO, et al. Clinical and molecular spectrum of somatic mosaicism in androgen insensitivity syndrome. Pediatr Res. 1999;46(6):684–90.
11. Mazur T. Gender dysphoria and gender change in androgen insensitivity or micropenis. Arch Sex Behav. 2005;34(4):411–21.
12. Meyer-Bahlburg HF. Gender monitoring and gender reassignment of children and adolescents with a somatic disorder of sex development. Child Adolesc Psychiatr Clin N Am. 2011;20(4):639–49.
13. Conn J, Gillam L, Conway GS. Revealing the diagnosis of androgen insensitivity syndrome in adulthood. BMJ. 2005;331(7517):628–30.

14. Hughes IA, Houk C, Ahmed SF, Lee PA, LWPES Consensus Group, ESPE Consensus Group. Consensus statement on management of intersex disorders. Arch Dis Child. 2006;91(7):554–63.

15. Cools M, Wolffenbuttel KP, Hersmus R, Mendonca BB, Kaprová J, Drop SLS, et al. Malignant testicular germ cell tumors in postpubertal individuals with androgen insensitivity: prevalence, pathology and relevance of single nucleotide polymorphism-based susceptibility profiling. Hum Reprod. 2017;32(12):2561–73.

16. Levin HS. Tumors of the testis in intersex syndromes. Urol Clin North Am. 2000;27(3): 543–51, x.

17. Hurt WG, Bodurtha JN, McCall JB, Ali MM. Seminoma in pubertal patient with androgen insensitivity syndrome. Am J Obstet Gynecol. 1989;161(3):530–1.

18. Chaudhry S, Tadokoro-Cuccaro R, Hannema SE, Acerini CL, Hughes IA. Frequency of gonadal tumours in complete androgen insensitivity syndrome (CAIS): a retrospective case-series analysis. J Pediatr Urol. 2017;13(5):498.e1–6.

19. Döhnert U, Wünsch L, Hiort O. Gonadectomy in complete androgen insensitivity syndrome: why and when? Sex Dev. 2017;11(4):171–4.

20. Hiort O, Holterhus PM. Androgen insensitivity and male infertility. Int J Androl. 2003;26(1):16–20.

21. Finlayson C, Johnson EK, Chen D, Dabrowski E, Gosiengfiao Y, Campo-Engelstein L, et al. Proceedings of the working group session on fertility preservation for individuals with gender and sex diversity. Transgend Health. 2016;1(1):99–107.

22. Batista RL, Costa EMF, Rodrigues AS, Gomes NL, Faria JA Jr, Nishi MY, et al. Androgen insensitivity syndrome: a review. Arch Endocrinol Metab. 2018;62(2):227–35.

23. Akin JW. The use of clomiphene citrate in the treatment of azoospermia secondary to incomplete androgen resistance. Fertil Steril. 1993;59(1):223–4.

24. Tordjman KM, Yaron M, Berkovitz A, Botchan A, Sultan C, Lumbroso S. Fertility after high-dose testosterone and intracytoplasmic injection in a patient with androgen insensitivity syndrome with a previously unreported androgen receptor mutation. Andrologia. 2014;46(6):703–6.

25. Pinsky L, Kaufman M, Killinger DW. Impaired spermatogenesis is not an obligate expression of receptor-defective androgen resistance. Am J Med Genet. 1989;32(1):100–4.

26. Weidemann W, Peters B, Romalo G, Spindler KD, Schweikert HU. Response to androgen treatment in a patient with partial androgen insensitivity and a mutation in the deoxyribonucleic acid-binding domain of the androgen receptor. J Clin Endocrinol Metab. 1998;83(4):1173–6.

27. Birnbaum W, Marshall L, Werner R, Kulle A, Holterhus P-M, Rall K, et al. Oestrogen versus androgen in hormone-replacement therapy for complete androgen insensitivity syndrome: a multicentre, randomised, double-dummy, double-blind crossover trial. Lancet Diabetes Endocrinol. 2018;6(10):771–80.

28. Al-Bassam A, Gado A. Feminizing genital reconstruction: experience with 52 cases of ambiguous genitalia. Eur J Pediatr Surg. 2004;14(3):172–8.

29. Baskin A, Wisniewski AB, Aston CE, Austin P, Chan Y-M, Cheng EY, et al. Post-operative complications following feminizing genitoplasty in moderate to severe genital atypia: results from a multicenter, observational prospective cohort study. J Pediatr Urol. 2020;16(5):568–75.

30. Callans N, De Cuypere G, De Sutter P, Monstrey S, Weyers S, Hoebeke P, et al. An update on surgial and non-surgical treatments for vaginal hypoplasia. Hum Reprod Update. 2014;20(5):775–801.

31. Chan JL, Levin PJ, Ford MP, Stanton DC, Pfeifer SM. Vaginoplasty with an autologous buccal mucosa fenestrated graft in two patients with vaginal agenesis: a multidisciplinary approach and literature review. J Min Invasiv Gynecol. 2017;24(4):670–6.

32. Shah R, Woolley MM, Costin G. Testicular feminization: the androgen insensitivity syndrome. J Pediatr Surg. 1992;27(6):757–60.

Chapter 2
Precocious Puberty

Nigel Pereira

Case

A 6-year-old African American girl is brought to the office by her mother. The girl began breast bud development 5 months ago. Pubic hair began to appear 4 months after the appearance of breast buds. Her mother has not observed any psychosocial or developmental milestone issues. The young girl appears well on examination with Tanner stage III breast development and stage II pubic hair growth. Her height has increased by 5 cm over the last 6 months. How does a physician approach this clinical presentation?

Background

Puberty in humans is a complex physiological process that is characterized by the transition from childhood to adulthood [1, 2]. Following the completion of puberty, young males and females are considered capable of reproducing [3, 4]. Puberty is marked by the maturation of external and internal genitalia, development of secondary sexual characteristics, acceleration of linear growth velocity, and in females, the beginning of menses (menarche) [5, 6]. For the purpose of this chapter, we will limit our discussion to female puberty.

The pubertal transition in females occurs due to two processes: gonadarche and adrenarche [6]. The former is associated with the growth and maturation of the ovaries which, in turn, results in increased sex steroid hormone synthesis, follicular development, and ovulation. The latter involves maturation of the adrenal cortex,

N. Pereira (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: nip9060@med.cornell.edu

thereby increasing adrenal androgen production, leading to the appearance of pubic hair (pubarche). The re-activation of the hypothalamic–pituitary–ovarian (HPO) axis is thought to drive gonadarche. Specifically, increased pulsatile gonadotropin-releasing hormone (GnRH) secretion is noted during puberty, which stimulates follicle-stimulating hormone (FSH) and luteinizing hormone (LH) synthesis, resulting in downstream ovarian activity and consequently breast development (thelarche). The mechanism of adrenarche remains unknown. Of note, the absence of adrenarche in humans does not seem to impact fertility or impede the process of gonadarche [6].

Normal Pubertal Stages

In general, puberty encompasses the following sequential events: accelerated linear growth, thelarche, adrenarche, and menarche. Population-based studies have indicated that African American girls begin puberty between age 8 and 9, while Caucasian girls do so by age 10 [5, 6]. However, thelarche and/or adrenarche can occur as early as age 6 and 7 in African American and Caucasian girls, respectively. Growth spurt generally occurs 2 years prior to thelarche and can be associated with a 6–11 cm increase in height within 1 year. This phase of linear acceleration is mediated by increasing estrogens from the ovary and the secretion of growth hormone from the pituitary gland. Gonadarche and adrenarche often occur concomitantly; however, there can be discordance between the two phases even in normal pubertal development. The changes during gonadarche and adrenarche are classified into five distinct stages called the Tanner stages, ranging from stage I (prepubertal) to stage V (adult) [3, 5, 6]. These stages have been summarized in Fig. 2.1 and Table 2.1. Menarche usually occurs about 2 years after thelarche. Menstrual cycles during the first 1–2 years are usually anovulatory and irregular, ranging between 21 and 45 days. However, within 3–5 years of menarche, most menstrual cycles are ovulatory and regular, varying between 21 and 35 days [5, 6].

Differential Diagnosis of Precocious Puberty

The most commonly accepted definition of precocious puberty is the development of secondary sexual characteristics before the age 8. It is known that a proportion of girls can attain secondary sexual characteristics before age 8. In such girls, clinical evaluation can be considered based on clinical presentation or the anxiety of parents. However, thelarche or adrenarche before age 6 should warrant immediate clinical evaluation [3, 4]. Precocious puberty occurs five times more often in females when compared to males, with an estimated incidence of 1 in 5000–10,000. The differential diagnoses of precocious puberty can be classified into two distinct categories: central precocious puberty (CPP) and peripheral precocious puberty (PPP)
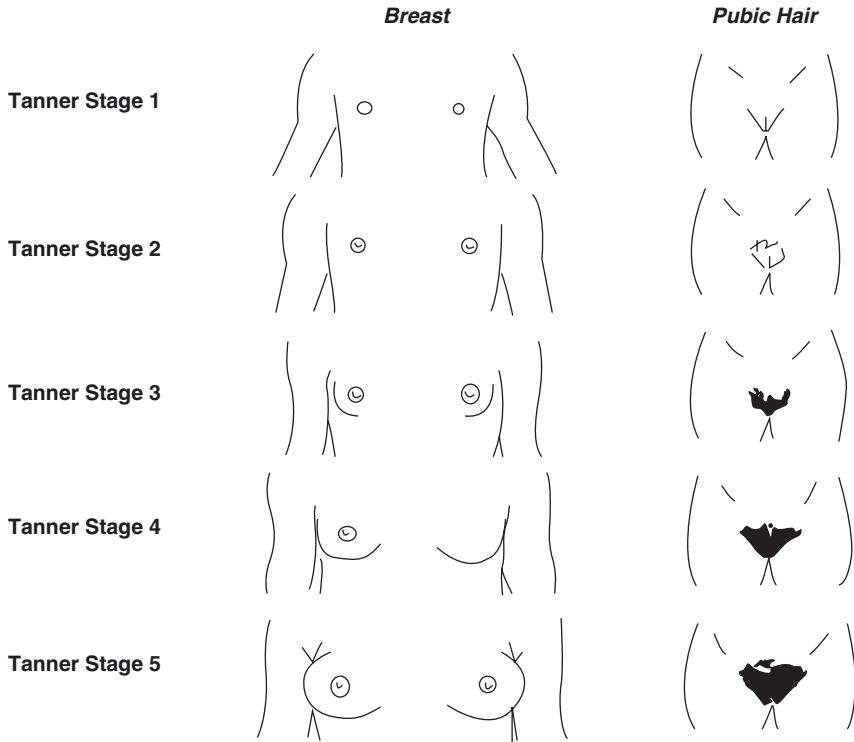
Fig. 2.1 Summary of Tanner stages, ranging from stage I (pre-pubertal) to stage V (adult)

Table 2.1 Summary of the Tanner stages of pubertal development

Tanner stage	Breast	Pubic hair
1	Pre-pubertal	No pigmented hair
2	Breast budding	Some pigmented, coarse hair over labia majora
3	Enlargement of breast and areolae	Extension of pigmented, coarse hair over mons pubis
4	Secondary mound of areolae	Nearing adult pattern
5	Mature contour	Adult pattern

[1]. CPP is also called GnRH-dependent precocious puberty, complete precocious puberty, or true precocious puberty. All these precocious puberty terms signify the same mechanism, i.e., premature activation of the HPO axis [3, 4]. Approximately, 80% of all precocious puberty cases are CPP, of which 75–90% are idiopathic in nature. PPP occurs when there is excessive secretion of sex steroids independent of the HPO axis [3, 4]. Therefore, PPP is also called GnRH-independent precocious puberty or incomplete precocious puberty. The source of sex steroids in PPP can be from exogeneous sources, tumors or genetic mutations. Table 2.2 summarizes the various causes of CPP and PPP [1, 3, 4].

Table 2.2 Non-exhaustive differential diagnoses of central and peripheral precocious puberty

Central precocious puberty
Idiopathic
Central nervous system tumors: Craniopharyngioma, astrocytoma, hypothalamic hamartoma, ependymoma, neurofibroma, luteinizing hormone-secreting adenoma
Central nervous system congenital malformations: Arachnoid cyst, hydrocephalus, myelomeningocele, suprasellar cyst, ectopic posterior pituitary lobe
Central nervous system acquires diseases: Inflammatory processes such as abscess, encephalitis, meningitis, sarcoidosis, radiation, trauma
Activating genetic mutations in the *KISS1R* and/or *KISS1* genes
Inactivating genetic mutations in the *MKRN3* gene
Peripheral precocious puberty
Adrenal tumors or congenital adrenal hyperplasia
Primary hypothyroidism
McCune-Albright syndrome
Endocrine disruptors
Ovarian cysts or tumors
Aromatase excess

Evaluation [1, 3, 4]

The evaluation of precocious puberty should always begin with a detailed clinical history, which should include the age of onset of each clinical symptom and progression of physical changes. A detailed pediatric and neonatal history should be noted. Emphasis should also be placed on any symptoms associated with central nervous system disease (CNS) such as personality changes, visual changes, polyuria, or polydipsia. Any history of CNS infectious, trauma, or accidental ingestion of hormonal contraception or supplementation should be elicited. A detailed family history should also be reviewed, including the age of puberty in parents, and/or any family members with a history of precocious puberty.

Physical examination begins with a detailed skin examination to look for any café-au-lait spots. The examination should also consist of measurements of linear growth velocity and secondary sexual characteristics as per Tanner staging. Heights should be plotted over time on a growth chart and should also be compared to midparental height. As noted earlier, thelarche is generally the first sign of puberty, and therefore, the presence of firm glandular tissue under the areolae is indicative of thelarche. Basic hormonal measurement should include FSH, LH, estradiol (E2), adrenal hormones, and thyroid function tests. Assessment of bone age with a radiograph of the left hand and wrist is recommended given that bone age is advanced in precocious puberty. In general, advancement of bone age of more than 1 year or 2 standard deviations from chronological age is considered significant. Magnetic resonance imaging (MRI) of the brain is generally warranted for all girls below age 6 years with suspected precocious puberty. Pelvic ultrasonography is important when precocious puberty due to ovarian cysts or tumors is suspected. Testing of
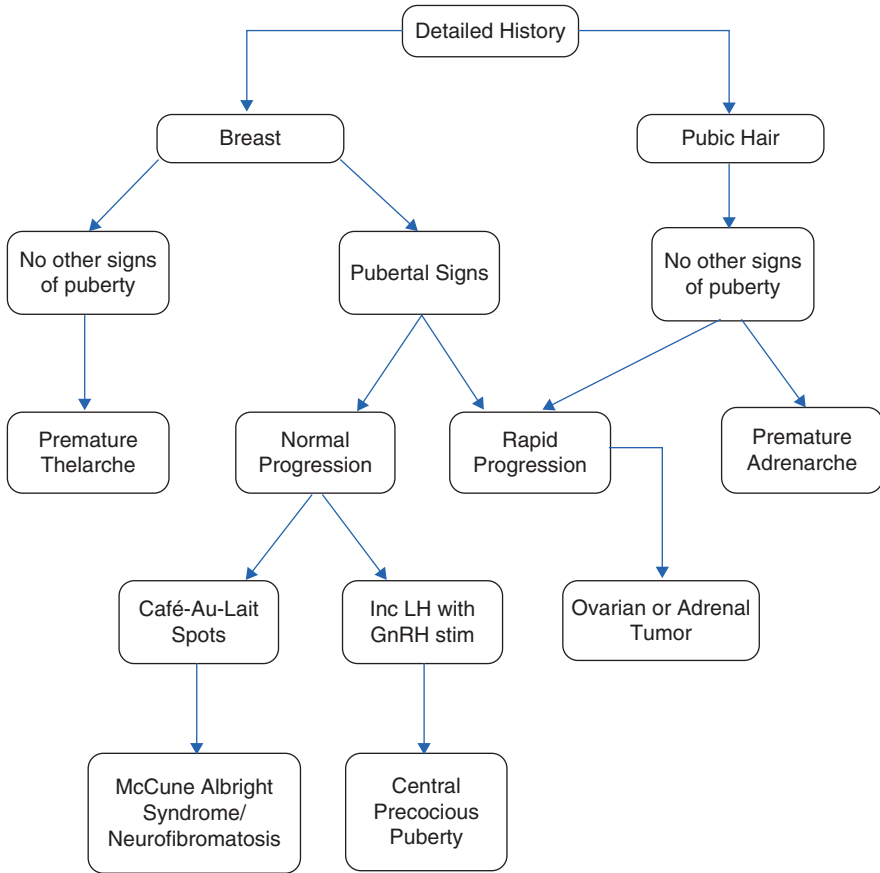
Fig. 2.2 A putative approach to investigate precocious puberty in young girls

MKRN3, *DLK1*, or *KISS1* mutations is generally reserved for the investigation of familial precocious puberty, defined by the occurrence of precocious puberty in more than one family member. Figure 2.2 summarizes a putative approach to investigate precocious puberty in young girls.

Clinicians should make every effort to distinguish true precocious puberty from isolated thelarche or adrenarche. Isolated thelarche is associated with the appearance of breast tissue without other signs of puberty. It is generally benign, self-limited and does not progress to precocious puberty. Isolated adrenarche is characterized by an increase in adrenal androgens, resulting in the development of axillary and pubic hair, as well as apocrine body odor, and acne. Isolated adrenarche is also benign in nature. Adrenal tumors can manifest with virilizing features such as clitoromegaly and increased pubic hair, as well as signs of glucocorticoid excess such as hypertension, weight gain, striae, and hirsutism. Due to the pulsatile nature of GnRH, random measurements of FSH or LH may provide limited information

about the type of precocious puberty; however, in general, suppressed FSH secretion in the presence of elevated sex steroids suggests the presence of PPP. Basal LH levels >0.6 IU/L have been used to rule out CPP, though with limited success. Therefore, in such cases, GnRH stimulation can be utilized to diagnose true CPP. A post-stimulation LH value of ≥5 IU/L after a 100-μg IV dose of GnRH is considered diagnostic for CPP in patients with pubertal signs.

Treatment [1, 2]

CPP

Long-acting GnRH-agonist (GnRH-a) is considered the gold standard for managing CPP. Administration of GnRH-a results in decreased FSH and LH levels, and consequently a significant decline in sex steroid levels. Occupation of GnRH receptors by long-acting GnRH-a administration results in desensitization and internalization of receptors, resulting in diminishing FSH and LH levels. Various formulations of long-acting GnRH-a are available, including intramuscular depot, subcutaneous injection, and subcutaneous implant. There is minimal agreement about the monitoring of hormonal parameters or ideal hormone levels during GnRH-a therapy. Thus, the overall goals of treatment are aimed toward the stabilization of pubertal progression, avoiding early menarche, decreasing premature advancement of bone age, and declining rapid increase in linear growth velocity. Studies have shown that girls who begin GnRH-a therapy before age 6 have the greatest benefit in terms of adult height, while those treated after age 8 have minimal benefit.

The most common side effects associated with GnRH-a treatment include headaches, hot flushes, and local injection-site reactions. Sterile abscesses may develop with intramuscular formulations or subcutaneous implants, limiting its efficacy. Furthermore, fracture of subcutaneous implants may occur during extraction, necessitating ultrasound-guided removal of the fragments. Vaginal bleeding may occur after the first dose of GnRH-a due to a transient increase in FSH, LH, and E2. Weight gain can also be a GnRH-a-associated side effect.

There is limited consensus about an optimal age to discontinue GnRH-a treatment. However, experts agree that it should be individualized to predicted height, synchronization of puberty with other sibling and/or peers, and the level of personal distress. Most often, treatment withdrawal is initiated at 12–12.5 years of bone age in girls. Menses spontaneously occur within 12 months of GnRH-a cessation.

PPP

The treatment of PPP depends on its etiology. For example, PPP symptoms due to congenital adrenal hyperplasia can be suppressed with glucocorticoids. Surgery is the standard of care for sex steroid-secreting tumors; however, surgery may not be required for E2-producing follicular cysts as they may regress spontaneously.

The treatment of McCune-Albright syndrome (MAS) deserves special mention. MAS is characterized by precocious puberty, abnormal fibrous tissue in bones called polyostotic fibrous dysplasia, and light brown patches of skin called café-au-lait spots. MAS is caused by an activating mutation of the α-subunit of the G-protein. PPP in MAS is caused by E2 synthesized from autonomously functioning ovarian cysts. Young MAS girls with frequent menses, rapidly progressing puberty, and accelerated growth and bone age normally benefit from treatment. Due to their binding to the cytochrome P450 portion of aromatase, aromatase inhibitors inhibit the conversion of androgens to estrogens, thereby forming the mainstay of PPP treatment in girls with MAS. Oral letrozole 2.5 mg daily is considered first-line for the management of PPP in MAS, whereas tamoxifen is used as second-line or for adjuvant therapy. Bisphosphonates can be used to treat persistent and/or moderate-to-severe bone pain. However, its impact on reducing the progression or size of fibrous dysplasia lesions is unknown.

It is important to note that CPP and PPP can occur concomitantly in children due to early activation of the HPO access. In such cases, additional GnRH-a treatment is indicated.

Discussion

Precocious puberty is a complex clinical entity, which can be distressing to young girls and their parents. Clinicians evaluating young girls with precocious puberty should aim to differentiate between isolated thelarche, isolated adrenarche, and precocious puberty. The former two are generally benign and self-limited, without onset of menses. Within the realm of precocious puberty, clinicians should focus their clinical history and investigation to distinguish between CPP and PPP. The majority of precocious puberty cases are CPP or GnRH-dependent, of which up to 90% of cases are idiopathic in nature. PPP or GnRH-dependent precocious puberty occurs when there is excessive secretion of sex steroids independent of the HPO axis. Administration of long-acting GnRH-agonist is the main therapeutic strategy in cases of CPP. In contrast, PPP requires suppression of sex steroids depending on its source.

References

1. Cheuiche AV, da Silveira LG, de Paula LCP, Lucena IRS, Silveiro SP. Diagnosis and management of precocious sexual maturation: an updated review. Eur J Pediatr. 2021;180(10):3073–87.
2. Schoelwer M, Eugster EA. Treatment of peripheral precocious puberty. Endocr Dev. 2016;29:230–9.
3. Sultan C, Gaspari L, Maimoun L, Kalfa N, Paris F. Disorders of puberty. Best Pract Res Clin Obstet Gynaecol. 2018;48:62–89.
4. Cantas-Orsdemir S, Eugster EA. Update on central precocious puberty: from etiologies to outcomes. Expert Rev Endocrinol Metab. 2019;14(2):123–30.
5. Speroff L, Fritz MA. Clinical gynecologic endocrinology and infertility. 7th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2005.
6. Strauss JF, Barbieri RL. Yen & Jaffe's reproductive endocrinology, Chapter 18. 7th ed. Philadelphia, PA: Elsevier; 2014.

Chapter 3
Delayed Puberty

Nigel Pereira

Case

A 14-year-old young girl presents to the office with her mother for the evaluation of delayed puberty. Her past pediatric and medical history is unremarkable. Physical examination reveals height of 140 cm, Tanner stage I breast development and pubic hair development. A webbed-neck and broad chest is noted. How does a physician approach this clinical presentation?

Background

Puberty is characterized by rapid physical and psychological changes which result in reproductive competence and the ability to conceive. The pubertal transition requires an intact hypothalamic–pituitary–ovarian (HPO) axis and is marked by increased pulsatility of gonadotropin-releasing hormone (GnRH). This in turn results in increased follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, thereby stimulating sex-steroid production by the ovaries.

N. Pereira (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: nip9060@med.cornell.edu

Definition of Delayed Puberty

The stages of normal puberty have been discussed in the Precocious Puberty chapter. In general, delayed puberty is defined as the absence of breast development in girls at an age that is 2–2.5 standard deviations later than the population mean. In the United States, this has been traditionally set at age 13 years for females. Population-based data have suggested a downward or earlier trend in female pubertal timing in the United States and some European countries. Therefore, some clinicians have proposed to revise the criteria with younger age cut-offs for certain countries, races, and/or ethnic groups. It is important to note that the development of pubic hair is not considered for the diagnosis of delayed puberty given that isolated pubarche can occur due to the adrenal glands, independent of the HPO axis.

As puberty involves estradiol (E2)-mediated increase in linear growth, delayed puberty can affect skeletal growth and bone mineralization. In fact, almost a third of total bone mineralization occurs within 4 years of puberty, and approximately 90% of peak skeletal bone mass is attained by age 18. Thus, delayed puberty causes inadequate circulating estradiol (E2), which can result in sub-optimal bone mineralization, and therefore, an increased risk of fracture. Although adult height can theoretically be compromised, most girls with delayed puberty have an adult height that is only slightly below their genetic potential. Delayed puberty may have a major impact on psychological health and has been shown to impact psychosocial outcomes for patients and their families. Some observational studies also suggest that delayed puberty can also hinder early academic success.

Differential Diagnosis of Delayed Puberty

A detailed discussion about the physiologic and genetic mechanisms underlying delayed puberty is beyond the scope of this chapter. However, it is important to note that delayed puberty may occur as an extreme variation of normal pubertal development. Such a developmental pattern is called constitutional delay of growth and puberty (CDGP). Current data suggest that up to 30–35% of girls with delayed puberty may have CDGP and therefore constitutes the most common form of delayed puberty. Although CDGP is common, it remains a diagnosis of exclusion, i.e., other forms of delayed puberty have to be ruled out. While the precise cause of CDGP remains unknown, it is thought to have a strong genetic predisposition, with the majority of patients reporting a family history of delayed puberty.

The other causes of delayed puberty can be classified into three different categories based on FSH and LH levels—hypergonadotropic hypogonadism, permanent hypogonadotropic hypogonadism, and transient hypogonadotropic hypogonadism. Hypergonadotropic hypogonadism is characterized by elevated FSH and LH levels due to gonadal dysfunction or failure. Primary ovarian insufficiency due to Turner's syndrome (45 X0) or XX gonadal dysgenesis is perhaps the most well-recognized

Table 3.1 Summary of differential diagnoses of delayed puberty

Delayed puberty type	Frequency (%)	Differential diagnoses
Constitutional delay	30–35	Constitutional delay of growth and puberty
Hypergonadotropic hypogonadism	25	Turner's syndrome; gonadal dysgenesis; chemoradiation
Permanent hypogonadotropic hypogonadism (congenital or acquired)	20	GnRH deficiency; Kallmann syndrome; combined pituitary hormone deficiency; central nervous system (CNS) tumors; infiltrative diseases of CNS; CNS chemoradiation
Transient hypogonadotropic hypogonadism	20	Eating disorders such as anorexia nervosa or bulimia; systemic diseases such as inflammatory bowel disease, celiac disease, hypothyroidism; excessive exercise

form of hypergonadotropic hypogonadism. Other causes include exposure to chemotherapy and/or pelvic radiation. Permanent hypogonadotropic hypogonadism is associated with persistently low FSH and LH levels owing to hypothalamic or pituitary disorders. Permanent hypogonadotropic hypogonadism can be divided into congenital or acquired causes. Idiopathic or isolated hypogonadotropic hypogonadism (IHH) is the most common congenital form of hypogonadotropic hypogonadism. Other congenital causes include genetic syndromes such as Kallmann syndrome, Prader-Willi syndrome, and Bardet-Biedl syndrome. Acquired hypogonadotropic hypogonadism can result from central nervous system (CNS) tumors, infiltrative disorders of the CNS such as histiocytosis, as well as CNS trauma, surgery or chemo-radiation. In contrast to permanent hypogonadotropic hypogonadism, transient hypogonadotropic hypogonadism, also called as functional hypogonadotropic hypogonadism, is due to delayed maturation of the HPO axis, secondary to an underlying medical condition. Common disorders associated with transient hypogonadotropic hypogonadism include stress, decreased caloric intake, hypothyroidism, inflammatory bowel disease, celiac disease, and diabetes. Bone marrow-related illnesses such as leukemia, thalassemia, and sickle cell disease are known to cause hypogonadotropic hypogonadism. Malnutrition due to eating disorders, excessive exercise, or underlying medical conditions can inhibit the HPO axis transiently, thereby resulting in delayed puberty. Table 3.1 summarizes the various differential diagnoses of delayed puberty.

Evaluation

History and Physical Examination

When evaluating any young girl with delayed puberty, a clinician must remember that the normal pubertal transition can vary significantly even among healthy girls. Thus, delayed puberty may not necessarily signify a medical or

developmental problem. Due to the vast differential diagnosis of delayed puberty, every effort should be made to elicit a detailed medical history, neonatal/pediatric history, as well as family history, including the age of puberty in parents, and/or any family members with a history of delayed puberty. Medical history should include questions about systemic diseases, including autoimmune diseases, as well as any CNS trauma, surgery or symptoms such as headaches, dizziness, or visual changes. Pertinent neonatal and pediatric exposures to chemoradiation should be noted. Patients and their parents should be asked about history of eating disorders, excessive exercise, and psycho-social functioning. Dysmorphic features or delayed cognitive development may indicate an underlying genetic syndrome.

Physical examination should include current and previous height, weight, body mass index (BMI), and linear growth velocity, which can be determined from growth charts. Clinicians should note any midline facial defects, neurologic deficits, including anosmia, and any signs of androgen excess. Features of Turner's syndrome such as short height, broad chest, webbed neck, and low hairline should be documented, if present. Tanner staging should be performed. Most often, examination of external genitalia may suffice; however, when needed, pelvic ultrasonography may be performed to evaluate internal pelvic structures.

Laboratory Investigation and Imaging

Every effort should be made to rule out other forms of delayed puberty before achieving the diagnosis of CDGP. A basic initial evaluation of delayed puberty includes measurements of FSH, LH, E2, and a single radiograph of the left hand and wrist to determine bone age. LH and E2 levels of >0.3 mIU/mL and 20 pg/mL, respectively, suggest onset of puberty. Young girls with CDGP can have a bone age that is delayed by about 2 years compared to chronological age. It may be challenging to distinguish between CDGP and IHH in routine testing as both are due to deficiency of FSH and LH secretion. In fact, there is considerable overlap of FSH and LH values in girls with CGDP and IHH. The GnRH stimulation test has limited value in such situations, given significant variability in response, and the lack of a commonly accepted cut-off value. However, a history of delayed puberty in a parent or sibling followed by spontaneous onset of puberty supports a diagnosis of CDGP. In contrast, IHH leads to permanent pubertal delay due to the absence of GnRH secretion. In such cases, a family history of pubertal development with exogenous sex steroids can support the diagnosis of IHH.

Advanced laboratory testing or imaging is dictated by the patient's history, physical examination or FSH, LH, and E2 levels. For example, CNS symptoms or headaches may warrant magnetic resonance imaging (MRI) of the brain and/or pituitary. History of unintentional weight loss may require evaluation of thyroid function tests

and assessment of auto-immune or inflammatory bowel conditions. Excessive exercise or restrictive food behavior may prompt biochemical testing and psychological counseling. Finally, elevated FSH levels may require a karyotype.

Treatment

Treatments for delayed puberty aim to optimize age-appropriate pubertal development, height and peak bone mass. A significant proportion of girls with CDGP can be managed expectantly, given that pubertal progression will occur with time. However, treatment to induce puberty maybe initiated to reduce psychosocial difficulties and anxiety related to growth and body habitus when compared to peers. In other words, treatment is aimed at achieving sexual maturation similar to peers. Generally, a very low dose of estrogen is initiated, which is gradually increased over a 3–4-year period until maintenance dosing is achieved. The target E2 level in such girls is between 50 and 150 pg/mL. Most experts agree that girls with CDGP or transient hypogonadotropic hypogonadism can be treated with an initial 4–6-month course of estrogen therapy, followed by discontinuation and reassessment of pubertal progression. Girls with hypergonadotropic hypogonadism or permanent hypogonadotropic hypogonadism require cyclic progestin therapy along with estrogen therapy to allow endometrial cycling. Common estrogen formulations include ethinyl estradiol, which is a component of oral contraceptive pills (OCPs), oral or transdermal 17β estradiol, and conjugated equine estrogens. Some adolescents may prefer OCPs given its convenience and use among other peers. However, OCPs are rarely used for maintenance therapy. OCPs may also have a higher risk of thromboembolism. Following induction of puberty, most patients can continue oral or transdermal estrogen therapy with cyclic progestin or may use combined estrogen and progestin compounds for maintenance therapy. Unfortunately, randomized controlled trials comparing various estrogen formulations for the treatment of delayed puberty are currently lacking. Novel treatments for delayed puberty such as kisspeptin, gonadotropins, GnRH, and GnRH-agonists are still considered investigational.

Discussion

Delayed puberty can be distressing for young girls and their parents. Certain forms of delayed puberty such as CDGP can occur as a variation of normal pubertal development. Although CDGP represents the most common form of delayed puberty, clinicians should make a diligent effort to rule out other causes of delayed puberty with meticulous history and physical examination. A basic initial evaluation of

delayed puberty utilizing measurements of FSH, LH, E2 can distinguish between hypergonadotropic hypogonadism, permanent hypogonadotropic hypogonadism, and transient hypogonadotropic hypogonadism. Treatment strategies using estrogen and progestin aim to optimize pubertal development, height, and peak bone mass.

Suggested Readings

1. Trotman GE. Delayed puberty in the female patient. Curr Opin Obstet Gynecol. 2016;28(5):366–72.
2. Fenichel P. Delayed puberty. Endocr Dev. 2012;22:138–59.
3. Palmert MR, Dunkel L. Clinical practice. Delayed puberty. N Engl J Med. 2012;366(5):443–53.
4. Harrington J, Palmert MR. An approach to the patient with delayed puberty. J Clin Endocrinol Metab. 2022;107(6):1739–50.

Chapter 4
Primary Amenorrhea

Alexis Melnick

Case

A 16-year-old G0 is brought to her pediatrician by her mother who is concerned that she has not yet started menstruating. Both of her older sisters achieved menarche at 13 and her mother at 12. She reports breast development starting at the age of 11 and states that she has not needed to buy new bras for the past year—she currently wears a C-cup. She shaves her legs but has not yet needed to shave her underarms. She is on her high school soccer team and enjoys running during the off season. She denies any recent increase in physical activity or changes in weight or diet. She also denies any history of sexual activity.

Her past medical history and past surgical history are unremarkable other than tonsillectomy at age 9. She takes occasional Tylenol as needed and does not take any medications regularly. Her family history is significant for hypothyroidism in her mother and maternal aunt, and prostate cancer in her paternal grandfather. She is a non-smoker and denies alcohol and illicit drug use.

Physical exam reveals normal vital signs. Height is 5′3″ and BMI is 22 kg/m². A urine pregnancy test done in the office is negative. Breast development is Tanner Stage IV and pubic hair is Tanner Stage II. Examination of the thyroid reveals no enlargement or masses. Pelvic exam is significant for normal external female genitalia. The patient is unable to tolerate a speculum exam but on bimanual examination, no cervix is palpated and the vagina appears to be short. The patient is then referred for an abdominal ultrasound, which reveals absence of the uterus. Bloodwork results are significant for normal FSH, LH, TSH, and prolactin levels. Serum estrogen levels are on the low end of normal and testosterone levels are noted

A. Melnick (✉)

Reproductive Medicine and Ob/Gyn, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

e-mail: alm2036@med.cornell.edu

to be elevated, in the normal male adolescent range. Karyotyping is then performed revealing a 46,XY karyotype.

Given these findings, a diagnosis of complete androgen insensitivity syndrome is made. A subsequent MRI reveals intrabdominal testes. The patient and her family are referred to adolescent gynecology and for psychological counseling. After a thorough discussion with her gynecologist, the determination is made to wait until breast development is complete and then plan for laparoscopic gonadectomy (removal of the testes) and subsequent hormone replacement with estrogen. The patient is also given a set of vaginal dilators and instructed on proper use.

Discussion

Primary amenorrhea is defined by the absence of menarche by age 13 without secondary sexual characteristics or by age 15 with secondary sexual characteristics. Etiologies of primary amenorrhea can be characterized as gonadal dysgenesis, hypothalamic or pituitary disorders, anovulation, outflow tract abnormalities, and physiologic. The most common causes of primary amenorrhea are chromosomal abnormalities leading to gonadal dysgenesis, which accounts for approximately half of all cases. It is important to remember that all causes of secondary amenorrhea can also present as primary amenorrhea.

Evaluation of primary amenorrhea includes:

- Complete physical exam including:

  - Vital signs
  - Height
  - Weight
  - BMI calculation
  - Breast examination with Tanner staging
  - Skin examination (evidence of hirsutism, acne, striae, axillary/pubic hair with Tanner staging)
  - Thyroid exam
  - Genital and pelvic exam with focus on clitoral size, pubic hair development, intactness of the hymen, vaginal length, and presence of a cervix, uterus, and ovaries

- Laboratory evaluation including:

  - Urine or serum pregnancy test
  - TSH
  - Prolactin
  - Estradiol (E2), follicle-stimulating hormone (FSH), luteinizing hormone (LH)
  - Androgens
  - 17-OH Progesterone
  - Karyotype

- Imaging (if normal vagina and/or uterus are not present on initial examination):

  – Pelvic sonogram
  – Pelvic MRI

Differential Diagnosis

- Pregnancy
- Gonadal dysgenesis

  – 46,XX
  – 45,X (Turner Syndrome)
  – 46,XY (Swyer Syndrome) and other mixed karyotypes

- Anovulation

  – Polycystic ovary syndrome (PCOS)
  – Hyperprolactinemia
  – Thyroid disease

- Hypogonadotropic hypogonadism

  – Functional hypothalamic amenorrhea
  – Constitutional delay of puberty
  – CNS lesions (especially pituitary and hypothalamic disorders)
  – Gonadotropin-releasing hormone deficiency

- Anatomic

  – Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome
  – Androgen insensitivity syndrome
  – Outflow tract obstruction (imperforate hymen, transverse vaginal septum)

In working up a patient with primary amenorrhea, considering disorders based upon the level of control of the menstrual cycle (hypothalamus/pituitary, ovary, outflow tract) is a helpful approach.

- Hypothalamic and Pituitary Disease
  Hypothalamic causes of primary amenorrhea include functional hypothalamic amenorrhea and isolated GnRH deficiency. Tumors and infiltrative lesions of the hypothalamus and pituitary can also result in amenorrhea—these are often associated with elevated prolactin levels.

  – Functional hypothalamic amenorrhea is caused by suppression of the hypothalamic–pituitary axis due to chronic stress, weight loss with or without eating disorders, and excessive exercise. It is highly associated with the female athlete triad—a trio of menstrual dysfunction, low energy availability, and decreased bone mineral density. Functional hypothalamic amenorrhea is a

diagnosis of exclusion—in the absence of an obvious underlying cause, brain imaging (MRI) should be performed to rule out a central nervous system (CNS) lesion. Bone mineral density testing is also an important consideration, particularly in a patient with a history of more than 6 months of amenorrhea, severe nutritional deficiency, or stress fractures. Patients typically present with low-normal LH and FSH levels and low serum estradiol. Treatment is focused on correction of the underlying cause in order to restore normal function of the hypothalamic–pituitary–ovarian axis, and in turn, normal ovulation.

– Isolated GnRH deficiency, a rare cause of primary amenorrhea, is caused by failure or proper migration or development of GnRH neurons. Kallmann syndrome is due to failure of GnRH neuron migration and is associated with anosmia. Several genes have been implicated in GnRH deficiency and can be inherited in an autosomal dominant, autosomal recessive, or X-linked fashion. The majority of Kallmann syndrome cases are due to mutations in the *Kal1* gene and are inherited in an X-lined fashion. Patients present with low serum gonadotropin concentrations and low serum estradiol, and the clinical picture is difficult to distinguish from constitutional delay of puberty. Treatment consists of induction of puberty followed by lifelong hormone replacement therapy with both estrogen and progesterone. Fertility can be readily achieved by administration of exogenous gonadotropins, preferably preparations of FSH and LH combinations.

– Hyperprolactinemia can cause amenorrhea by disrupting the pulsatile secretion of GnRH, leading to disruption of FSH and LH secretion. It is a common cause of secondary amenorrhea but can rarely be associated with primary amenorrhea, often accompanied by galactorrhea (milky breast discharge). Since prolactin can be increased after eating, a fasting level should always be drawn after an initial elevated value prior to initiating a full workup. Once the elevation is confirmed, a brain MRI should be performed. The most common finding on MRI associated with amenorrhea is a prolactin secreting pituitary adenoma. Other non-structural causes of hyperprolactinemia are medication use (e.g., antipsychotics), hypothyroidism, and pregnancy. The management of hyperprolactemia is discussed in the chapter Hyperprolactinemia.

– Constitutional delay of puberty is characterized by both delayed adrenarche and gonadarche and is difficult to distinguish from congenital GnRH deficiency. It is five times more common in boys than girls and as such should be considered a diagnosis of exclusion. Often, medical history will reveal a family history of delayed puberty. Patients will go on to have completely normal pubertal development at a later age [1–3].

• Gonadal Dysgenesis

Primary amenorrhea is most commonly caused by chromosomal or genetic abnormalities leading to gonadal dysgenesis. Given either partial or complete lack of ovarian function, patients will present with elevated FSH levels and low serum estradiol levels. Treatment consists of pubertal induction followed by lifelong hormone replacement therapy.

- – Turner syndrome (45,X gonadal dysgenesis) is due to the absence of one X chromosome in all (55–60%) or some cells (mosaic Turner syndrome). It is the most common chromosomal cause of gonadal dysgenesis. Accelerated apoptosis of ovarian follicles leads to ovarian insufficiency, typically beginning in utero. However, some patients, particularly those with mosaic Turner syndrome, may have some residual ovarian function and may start and even complete puberty. Because external and internal female genital development is not dependent on estrogen action, the external female genitalia, uterus, cervix, and fallopian tubes develop normally. Short stature is the most common phenotypic characteristic. Other clinical features include a "shield" chest with the appearance of widely spaced nipples, a short and webbed neck, cubitus valgus, congenital lymphedema of the hands and feet, webbed neck, nail dysplasia, narrow and high-arched palate, and short fourth metacarpals and/or metatarsals. Patients may also present with skeletal, cardiac, and renal anomalies, and diagnosis is often made prior to expected age of puberty given these hallmark phenotypic findings.
  - – Swyer Syndrome (46,XY gonadal dysgenesis) is a rare cause of primary amenorrhea and can be caused by a variety of genetic mutations, although a clear genetic etiology is not identified in the majority of cases. The streak gonad fails to produce either AMH or testosterone leading to regression of the Wolffian ducts and development of the Mullerian ducts. As such, patients present with normal external and internal female genitalia and primary amenorrhea with absent secondary sexual characteristics. Karyotype is 46,XY. Because of the presence of a normal uterus and cervix, pregnancy can be achieved via oocyte donation [1–3].

- Polycystic Ovarian Syndrome

  Polycystic ovarian syndrome (PCOS) typically presents with either oligo- or secondary amenorrhea but may present with primary amenorrhea. Patients who present with primary amenorrhea have higher circulating androgen levels and incidence of obesity and are at higher risk of metabolic syndrome in the future. PCOS is a diagnosis of exclusion, and given it is not commonly seen in cases of primary amenorrhea, all potential etiologies should be ruled out in these patients. The diagnosis and management of PCOS is discussed further in its own chapter.

- Outflow Tract Abnormalities

  Congenital abnormalities of the female reproductive tract account for ~20% of cases of primary amenorrhea. Patients present with normal serum estradiol and gonadotropin levels (given their functional HPO axis) and normal secondary sexual characteristics. Cases involving obstruction of the reproductive tract (imperforate hymen, transverse vaginal septum) may present with cyclic pelvic pain often occurring at the expected time of menses. If left untreated, retrograde menstruation can lead to pelvic endometriosis due to the accumulation of menstrual blood in the uterus and tubes. Expeditious surgical correction of obstructive abnormalities is crucial.

– Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome refers to congenital absence of the vaginal and uterus, although some females may exhibit variable degrees of uterine development. It is caused by either agenesis or hypoplasia of the Mullerian duct system. Clinical presentation is variable and depends on the degree of agenesis. Women with a normal, obstructed uterus and those with a rudimentary uterine horn with functional endometrium may present with cyclic pelvic pain and/or a painful pelvic mass. While there are some genetic mutations associated with MRKH, the majority of cases are sporadic. Imaging studies can help to clarify the nature of the agenesis (vaginal, cervical, uterine) and to differentiate it from imperforate hymen and transverse vaginal septum. Treatment consists of creation of a functional vagina, either through mechanical dilation or surgery as well as removal of an obstructed rudimentary horn. Given normal ovarian function, patients can undergo ovarian stimulation and oocyte retrieval and achieve pregnancy through embryo transfer into a gestational carrier.

For our patient, the diagnosis of complete androgen insensitivity syndrome (CAIS) is in the category of outflow tract obstruction. It is an X-linked recessive disorder in which 46,XY patients present with a female phenotype. It is due to a defect in the androgen receptor, which renders cells resistant to the action of testosterone. The testes are functional—testosterone is made in normal quantities by Leydig cells, but due to the receptor defect, it is unable to act on cells and patients fail to develop all testosterone-dependent male sexual characteristics. This process starts in utero. Failure of the androgen receptor to recognize testosterone action leads to regression of the Wollfian ducts, while anti-Mullerian hormone (AMH), produced by Sertoli cells, leads to regression of the Mullerian ducts and its structures (the fallopian tubes, uterus, and upper third of the vagina). Given the dependence of testicular descent on testosterone action, the testes are typically located in the abdomen or inguinal region. At puberty, given the aromatization of testosterone to estrogen, normal breast development occurs. Axillary and pubic hair are scant given the lack of androgen action. The diagnosis is based upon the absence of the upper vagina, uterus, and fallopian tubes on physical exam and pelvic ultrasound, a male (46,XY) karyotype, and high serum testosterone concentrations (in the normal male range).

The differential diagnosis in a female presenting with primary amenorrhea, absent uterus, and a blind vaginal pouch includes Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome. However, these women can be distinguished from those with CAIS by the presence of normal axillary and pubic hair, a 46,XX karyotype, and serum estrogen and testosterone concentrations in the normal female range. Partial androgen insensitivity syndrome is associated with less severe receptor defects than those seen in CAIS and can present with partial virilization and ambiguous genitalia (refer to chapter Ambiguous Genitalia).

Management of CAIS is focused on the creation of a functional vagina, psychological treatment for both the patient and her family, and reduction in the risk of malignancy by properly timed gonadectomy. Leaving the functional testes in place

until completion of puberty allows for a smoother pubertal transition since testosterone is converted to estrogen. Because cryptorchidism is a risk factor for development of malignant germ cell tumors, once puberty is complete, the testes should be removed and hormonal replacement therapy started. Given the absence of a uterus in women with CAIS, estrogen therapy alone is sufficient. In women with partial AIS, gonadectomy should not be delayed given the risk of progressive virilization [1–3].

References

1. Welt CK, Barbieri RL. Causes of primary amenorrhea. In: Post TW, editor. Uptodate 2019. Accessed 1 May 2021.
2. Klein DA, Paradise SL, Reeder R. Amenorrhea: a systematic approach to diagnosis and management. Am Fam Physician. 2019;100(1):39–48.
3. Practice committee of the American Society for Reproductive Medicine. Current evaluation of amenorrhea. Fertil Steril. 2006;86:S148.

Chapter 5
Secondary Amenorrhea

Pietro Bortoletto and David Reichman

Case

A 34-year-old nulligravida with an 8-month history of secondary amenorrhea presented for evaluation. She reported menarche at age 12 with irregular cycles of 45–60 days intervals. In college she reported acne on her face and chest as well as dark hair growth on her chin and lower abdomen. Given her irregular cycles, she was recommended a levonorgestrel releasing intrauterine device (IUD) which improved her bleeding pattern but did not improve her hair growth or acne. After 5 years of IUD-induced amenorrhea, she discontinued her IUD 8 months prior to presentation but had not experienced any bleeding, spotting, or premenstrual symptoms. She denied excessive exercise, recent weight change, nipple discharge, hot flushes, or vision changes. While she was not interested in conceiving at the time of presentation, she expressed significant distress regarding the absence of her menses, acne, and hair growth.

Her past medical history is significant for migraines without aura. She has no surgical history and uses a topical retinoid to manage her acne and sumatriptan for migraines. She has never been pregnant before and her gynecologic history is unremarkable. Her family history is significant for non-insulin-dependent types 2 diabetes and hyperlipidemia. She is a non-smoker, denies illicit drug use, and uses alcohol socially.

On physical exam she was noted to be normotensive with a BMI of 23 kg/m^2, there was acne along her face and chest, and thick dark hair along her chin and lower abdomen. There was no evidence of striae, acanthosis nigricans, galactorrhea, or stigmata of thyroid disease. As part of her evaluation, a pelvic ultrasound was performed and revealed an unremarkable, anteverted uterus with a 12-mm

P. Bortoletto · D. Reichman (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: der2005@med.cornell.edu

endometrial stripe, and an antral follicle count of 46 without evidence of ovarian cysts. Laboratory evaluation revealed a negative serum hCG, TSH of 2.8 mIU/L, and prolactin of 4 ng/mL. Random follicle-stimulating hormone (FSH) was 4.2 mIU/mL, luteinizing hormone (LH) was 8.1 mIU/mL, and estradiol (E2) was 67 pg/ml. An anti-Müllerian hormone returned at 4.7 ng/mL, 17-hydroxyprogesterone was 90 ng/dL, and a total serum testosterone was 110 ng/dL.

Given her menstrual pattern, ultrasound findings, and serum testing, she was diagnosed with polycystic ovary syndrome. As she was not interested in conceiving at this time but still reported acne and hair growth, she was recommended a combined oral contraceptive pill to control her menstrual cycle and symptoms of hyperandrogenism.

Discussion

Secondary amenorrhea is defined as the absence of menses for more than 3 months in women with regular menstrual cycles or 6 months in those with irregular cycles. In adult patients, the most common cause of secondary amenorrhea is pregnancy followed by hypothalamic (35%), ovarian (40%), and pituitary (17%) disorders [1]. Once pregnancy has been ruled out, a stepwise approach to evaluate each level of the hypothalamic–pituitary–ovary (HPO) axis is essential for effective diagnosis and treatment (Table 5.1).

The first step in the assessment of a patient presenting with secondary amenorrhea is a thorough review of patient's behaviors and symptoms. Being that functional hypothalamic amenorrhea is the most common cause of secondary amenorrhea, assessment of recent changes in weight, diet, and exercise pattern is essential. Acute weight loss of as little as 10% below ideal body weight in addition to excessive exercise can precipitate a decrease in pulsatile GnRH secretion leading to amenorrhea [2]. Luckily, women with hypothalamic amenorrhea due to a clear precipitating factor such as weight loss are more likely to have return of their menses with behavioral modification [3]. Independent of weight loss and exercise, diets with severe restriction in fat consumption have also been shown to be associated with hypothalamic amenorrhea [4]. Probing of hypothalamic–pituitary disorder specific symptoms such as headaches, visual field changes, galactorrhea, and excessive thirst/urination allows for isolation of a likely HPO axis level to further evaluate. Downstream complaints of amenorrhea-induced estrogen deficiency such as hot flushes, vaginal dryness, and mood changes should also be assessed.

Second, it is important to thoroughly assess the patient's medical, surgical, and reproductive histories as well as medications for potential contributing causes. Systemic illness such as type 1 diabetes mellitus, Celiac disease, Cushing's disease, and thyroid disorders are commonly associated with changes in menstrual pattern and in many cases amenorrhea may be a presenting feature of the disease [5]. A recent history of cranial or pelvic surgery or radiation may raise suspicion for

Table 5.1 Common causes of secondary amenorrhea

	Causes
Hypothalamus	Functional hypothalamic amenorrhea
	Intracranial lesions
	Traumatic brain injury
	Infiltrative or inflammatory disease
Pituitary	Hyperprolactinemia
	Intracranial lesions
	Empty Sella syndrome
	Pituitary apoplexy or infarct
	Pituitary tumors
Ovary	Primary ovarian insufficiency
	• Gonadotoxic medication or radiation therapy
	• Fragile X premutation
	• Chromosomal abnormalities (i.e., Turner's syndrome)
	• Autoimmune disease
	• Iatrogenic (i.e., surgery)
Other	Pregnancy
	Thyroid dysfunction
	Adrenal disease
	Systemic illness (i.e., diabetes, Celiac)
	Polycystic ovary syndrome
	Cervical stenosis
	Intrauterine adhesions

treatment-related complications such as pituitary infarct, intrauterine adhesions, cervical stenosis, or premature ovarian failure. Obstetric history should be assessed for postpartum hemorrhage, retained products of conception, endometritis, and inability to breast-feed postpartum. Finally, medications that act on the HPO axis such as dopamine receptor antagonists, antipsychotics, and selective serotonin reuptake inhibitors must be evaluated as they may potentiate amenorrhea secondary to hyperprolactinemia.

The focus of the physical examination should be on assessing downstream stigmata of hypothalamic–pituitary–ovarian axis disease. A thorough neurologic and ophthalmologic exam can reveal symptoms of intracranial lesions or hypothalamic–pituitary process such as prolactinoma and inflammatory or infiltrative diseases that may impact GnRH secretion. Systemic signs of thyroid disease such as hair loss, goiter, dry skin/nails, and fatigue may be more easily identifiable as patients may often present with these concurrent complaints. A breast and pelvic exam allows for the assessment of galactorrhea as well as evidence of hypoestrogenism and outlet obstruction such as cervical stenosis. Importantly, targeted evaluation for swollen salivary glands, poor dentition, and callused knuckles should be considered when there is suspicion for disordered eating [6].

The laboratory evaluation for secondary amenorrhea follows a stepwise framework from common to less frequent causes. After pregnancy has been ruled out via serum or urine hCG, the following serum hormones should be evaluated: prolactin, TSH, FSH, and estradiol. Prolactin levels are considered

abnormal if above 40 ng/mL and values in the 20–40 ng/mL should be repeated while the patient is fasting. An abnormal prolactin level should prompt further evaluation of iatrogenic causes (i.e., medications) as well magnetic resonance imaging to evaluate for intracranial lesions (refer to Chap. 11). TSH values that are above 4.5 mIU/L or below 0.5 mIU/L warrant further evaluation of serum T3 and T4 and targeted workup for thyroid disorder. An FSH value greater than 15 mIU/L coupled with hypoestrogenism (hypergonadotropic hypogonadism) may suggest a problem at the level of the ovary such as primary ovarian insufficiency (POI). Suspicion for POI should be followed-up with additional testing to rule out karyotypic abnormalities such as Turner syndrome or fragile X premutation. Patients with low or normal FSH values coupled with hypoestrogenism can be classified as having hypogonadotropic hypogonadism and can be considered to have functional hypothalamic amenorrhea when systemic illness or other HPO disorders have been ruled out.

In patients with normal prolactin, TSH, FSH, and estradiol levels, such as the case we presented, a search for clinical and laboratory signs of hyperandrogenism should occur. Evidence of hirsutism, male pattern balding, acne, or virilization should warrant evaluation of serum androgens with a total testosterone and 17-hydroxyprogesterone (17-OHP). Elevated total testosterone or 17-OHP should prompt evaluation for androgen-secreting tumors, ovarian hyperthecosis, and congenital adrenal hyperplasia by a specialist.

Finally, in patients with normal serum testing and without signs of hyperandrogenism dynamic testing of the uterus and outflow tract should be considered. When pelvic ultrasound is available, a thickened endometrial stripe may serve to demonstrate sufficiently high estradiol levels. Oral progestins can then be used to withdraw the lining. In an appropriately estrogenized and proliferative endometrium with a patent outflow tract bleeding should occur within 2 weeks following cessation of an oral progestin. If endometrial lining is thin on pelvic ultrasound which is a concern for hypoestrogenism, an oral estrogen has to be added to effect bleeding. Should bleeding not occur, evaluation for cervical stenosis and intrauterine pathology should be pursued. In-office attempts at cannulation of the cervix and distending the uterine cavity via saline infusion sonogram can provide valuable information about potential for a surgical treatment for adhesive disease.

In summary, secondary amenorrhea is a common clinical scenario with a myriad of causes and inciting factors. An understanding of what the common and less common etiologies allows for a stepwise approach to evaluation. A thorough review of a patient's symptoms and history is often the most important clue to isolate where along the hypothalamus–pituitary–ovary axis a defect may occur. Thoughtful physical examination and targeted laboratory testing will further strengthen the diagnosis and allow for appropriate treatment to be undertaken.

References

1. Reindollar RH, Novak M, Tho SP, McDonough PG. Adult-onset amenorrhea: a study of 262 patients. Am J Obstet Gynecol. 1986;155(3):531–43.
2. Roberts RE, Farahani L, Webber L, Jayasena C. Current understanding of hypothalamic amenorrhoea. Ther Adv Endocrinol Metab. 2020;11:2042018820945854.
3. Perkins RB, Hall JE, Martin KA. Aetiology, previous menstrual function and patterns of neuro-endocrine disturbance as prognostic indicators in hypothalamic amenorrhoea. Hum Reprod. 2001;16(10):2198–205.
4. Couzinet B, Young J, Brailly S, Le Bouc Y, Chanson P, Schaison G. Functional hypothalamic amenorrhoea: a partial and reversible gonadotrophin deficiency of nutritional origin. Clin Endocrinol. 1999;50(2):229–35.
5. Klein DA, Paradise SL, Reeder RM. Amenorrhea: a systematic approach to diagnosis and management. Am Fam Physician. 2019;100(1):39–48.
6. Harrington BC, Jimerson M, Haxton C, Jimerson DC. Initial evaluation, diagnosis, and treatment of anorexia nervosa and bulimia nervosa. Am Fam Physician. 2015;91(1):46–52.

Chapter 6
Mullerian Anomaly

Samantha M. Pfeifer

Case

A 14-year-old female presents to the emergency room with severe pain that started with the onset of her menstrual cycle. She had menarche at age 13, and her menses have been occurring approximately every 5–6 weeks since then. Each time she has her menstrual cycle, she describes bleeding for 4–5 days with severe cramping located on the right side. Her dysmenorrhea has been increasing with each menstrual cycle. She started bleeding 2 days ago and is now writhing on the bed in severe right lower quadrant pain with some nausea. The pain radiates all over the right side. She is otherwise healthy. She had recurrent ear infections as a child, which have resolved. She is on no medications and has no allergies. She is in ninth grade and is doing well in school. Her mother reports that prenatal ultrasound during the pregnancy with SP revealed right renal agenesis. She denies renal or urinary issues. In the ER her vital signs are stable, and her abdomen is tender on the right with a mass noted in the right lower quadrant. Transabdominal ultrasound revealed a 14 × 8 cm fluid-filled mass in the right lower quadrant.

Differential Diagnosis

The presenting symptom in this young female is dysmenorrhea that is increasing in severity. One would consider primary or secondary dysmenorrhea. However, her pain is not generalized as is seen with primary dysmenorrhea, but rather localized on the right side. In addition, her history is notable for congenital right renal agenesis. Congenital

S. M. Pfeifer (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
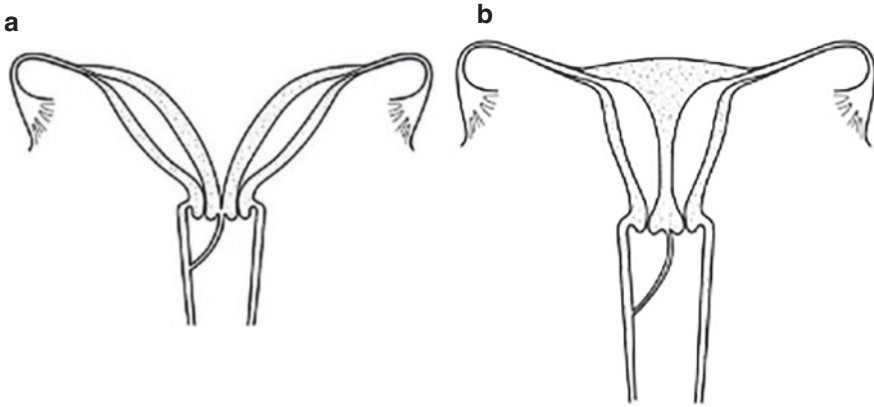e-mail: spfeifer@med.cornell.edu

Fig. 6.1 (**a**) Uterus didelphys, obstructed right hemivagina. (**b**) Complete septate uterus with obstructed right hemivagina. (Figures from: Pfeifer SM, Attaran M, Goldstein J, Lindheim S, Petrozza J, Rackow B, Zuckerman A, Siegelman E, Troiano R, Winters T, Ramaiah SD. ASRM Mullerian anomalies classification 2021. Fertil Steril 2021;116:1238–52)

renal anomalies are seen in association with Mullerian anomalies. In particular, congenital renal agenesis is associated with ipsilateral obstructed Mullerian anomalies [1–3]. Ultrasound imaging in this patient shows a fluid-filled mass in the right lower quadrant. Differential diagnosis of this mass includes a large right ovarian cyst, obstructed Mullerian anomaly including imperforate hymen, transverse vaginal septum, cervical agenesis, and obstructed hemivagina associated with uterus didelphys or obstructed right uterine horn. Given that this patient has been experiencing menstrual bleeding, complete obstructive anomalies such as imperforate hymen, transverse vaginal septum, and cervical agenesis are excluded. The increasingly severe right-sided dysmenorrhea, right renal agenesis, and imaging showing a large cystic mass are most consistent with obstructed right hemivagina (Fig. 6.1). The vagina is distensible and able to accommodate a large volume of menstrual blood and will present with a large mass. An obstructed uterine horn is less distensible than the vagina as it is smooth muscle and therefore unlikely to accommodate a large volume of menstrual blood or distend to the size noted on the ultrasound image. An ovarian cyst is possible but not likely to cause pain only during menstruation unless the cyst was endometriosis. An endometrioma cyst this size is atypical in such a young individual unless caused by an obstructed Mullerian anomaly.

Evaluation

Evaluation in this patient should focus on determining if there is an obstructed Mullerian anomaly, and if so where the level of obstruction is, and assessing the structure of the reproductive organs. The new ASRM Mullerian Anomaly Classification published in 2021 is a novel classification that utilizes words to name each anomaly, and in addition, it is an innovative tool that may be used to view the
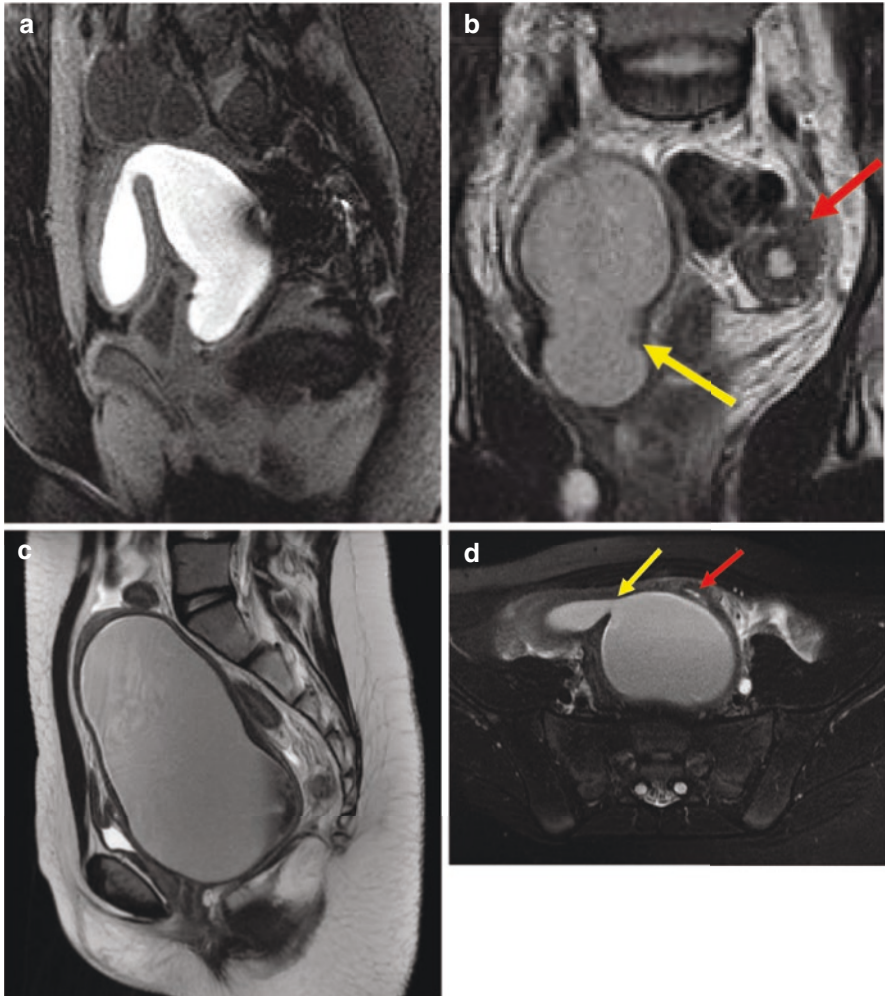
Fig. 6.2 (**a**) Sagittal T2-weighted MRI image showing obstructed hemivagina with hematocolpos, hematometra, and distended but well-defined cervix. (**b**) Coronal T2-weighted MRI image showing right hematometra and hematocolpos (yellow arrow) with left uterine horn cross section with normal endometrium (red arrow). (**c**) Sagittal T2-weighted MRI image of large obstructed hemivagina. (**d**) Axial T2-weighted MRI image showing distended right hemivagina with hematometra with well-defined right cervix (yellow arrow) and compressed left hemivagina adjacent to distended right hemivagina (red arrow). (Images from Samantha M. Pfeifer MD)

wide range of Mullerian anomalies and compare and contrast clinical presentation and imaging studies in order to arrive at the most likely diagnosis [4]. Magnetic resonance imaging (MRI) is the imaging modality preferred in these cases as the detail of the imaging in many imaging planes is able to define the anatomy (Fig. 6.2) [5]. 3D ultrasound imaging, although successful in differentiating uterine anomalies such as arcuate, septate, bicornuate, and didelphys, is not as good as MRI for the complex anomalies. When utilizing radiology studies to confirm diagnosis of

Mullerian anomalies, it is important to work with radiologists who are familiar with these anomalies, as these are rare, and many may not have experience in interpreting the studies. The images are best obtained parallel to the long axis of the uterus. This may be accomplished with the usual sagittal, transverse, and coronal planes, but may require additional imaging in the oblique transverse or coronal planes to best define the anatomy. In some cases, contrast gel may be inserted into the patient's vagina to define vaginal and cervical anatomy. When imaging complex anomalies, it is critical to work with the radiologist and discuss the differential diagnosis and suspected anomaly before performing the study so that an optimal study may be planned. After the studies have been performed, it is ideal to review the images with the radiologist to better understand and define the anatomy.

Physical exam can be helpful in confirming the correct diagnosis. However, a pelvic exam in a young girl who has never had such an exam can be frightening and as a result limited. It may not be feasible to get a good look at the vagina and cervix due to a small hymenal opening. Some advocate a rectal exam, but this may also be invasive for a young female. Examination of the external genitalia may be helpful and with her cooperation may not be threatening. However, imaging is usually a better and less invasive way to determine the anatomy. When there is concern, an exam under anesthesia may be performed with little distress for the patient. In addition, other minimally invasive techniques such as vaginoscopy, hysteroscopy, and even fluoroscopy can be utilized at that time to better define the anatomy.

In this patient the MRI was performed, and it revealed a uterus didelphys with the left hemiuterus lying in the left pelvis with a normal cervix and vagina noted. The right hemiuterus was positioned high in the right pelvis with a normal cervix and a large right hematocolpos measuring 14 × 8 × 8 cm. The lower margin of the hematocolpos extended to 2 cm above the symphysis. Both ovaries were normal. The right and left fallopian tubes were not visualized. Right renal agenesis was confirmed; the left kidney was normal with a normal collecting system. The MRI confirmed the diagnosis of uterus didelphys and obstructed right hemivagina associated with right renal agenesis as identified in the ASRM MAC2021 [4]. This condition has many names, including obstructed hemivagina and ipsilateral renal agenesis (OHVIRA) [3] and Herlyn-Werner-Wunderlich syndrome (HWW) [6]. It is classified by the ESHRE-ESGE Classification system as U3 C2 V2 [7].

Uterus didelphys and obstructed hemivagina associated with ipsilateral renal agenesis is a rare condition, and no clear incidence has been determined, although it is seen less frequently than Mullerian agenesis (congenital absence of the uterus, cervix, and vagina). The cause has yet to be determined, but it is believed to represent disruption of embryologic development of the mesonephric and/or paramesonephric ducts. The obstruction may be complete or partial and occur at the level of the vagina or cervix. The obstruction is more common on the right, with an incidence of 52–67% as reported in small case series [8, 9]. Approximately 95% of the cases are associated with ipsilateral renal agenesis; other reported anomalies include dysplastic/hypoplastic kidney on the ipsilateral side, pelvic kidney, and contralateral duplicated ureter. A small proportion of cases have been described with normal renal anatomy.

Presenting symptoms differ with complete and partial obstruction. Median age at presentation is 14 years with a range of 10–29 years, although presentation after age 18 is rare with complete obstruction. With complete obstruction at the level of the vagina presenting symptoms are severe dysmenorrhea, pelvic pain (initially unilateral), chronic pelvic pain, acute urinary retention, and paravaginal mass. For individuals with a partial obstruction, presenting symptoms include prolonged menstrual bleeding or bleeding between menses, unilateral pain, leukorrhea (infected vaginal discharge), foul-odored vaginal discharge, and sepsis. There is often a significant delay in diagnosis of months to years in these young women, especially in those with partial obstruction. This delay in diagnosis can lead to complications from retrograde menstruation, which have been observed at laparoscopy and include endometriosis (37%), hematometra (37%), hematosalpinx (22%), and pelvic adhesions (10%) [8]. The sequalae of the delay in diagnosis includes subsequent removal of ovary and fallopian tubes, infertility, and pelvic pain, as well as emotional impact.

The configuration of the uterus seen with obstruction of hemivagina is most commonly uterus didelphys, occurring in approximately 72% of cases. However, it is not the only uterine configuration. The other anomalies seen and their incidence in one study include complete septate uterus (14%), bicornuate bicollis (13%), and uterus didelphys with unilateral cervical atresia in lieu of vaginal obstruction (6%) [2].

Treatment

Goals of treatment are to definitively correct the Mullerian anomaly in a one-stage procedure and optimize fertility. The size of the obstructed hemivagina is an important consideration when deciding on surgical management. If the distended hemivagina is large and in close proximity to the patent hemivagina, then the most straightforward approach is to create a communication between the two hemivaginas by resecting the vaginal septum. If the obstructed hemivagina is small or far away from the patent vagina, then resection of the vaginal septum carries a high risk of restenosis and consideration should be made to remove the hemi-uterus, cervix, and hemivagina on the obstructed side (Fig. 6.3). Surgical drainage of the hematocolpos is a temporizing measure and should only be employed to treat acute pain when surgical expertise to correct the anomaly is not available. Laparoscopic drainage may be safer and carry less risk than transvaginal drainage. Concern of transvaginal drainage is introduction of bacteria into the obstructed hemivagina with the development of pyometra and risk of sepsis. If drainage of hematocolpos is performed, then referral for definitive surgery is indicated. The patient may be placed on continuous combined oral contraceptives to suppress menses.

Transvaginal resection of the vaginal septum to treat double uterus with obstructed hemivagina is a relatively straightforward procedure. There is no need for simultaneous laparoscopy unless there is evidence of significant upper tract disease on imaging. It is important to have accurate imaging to define the size and
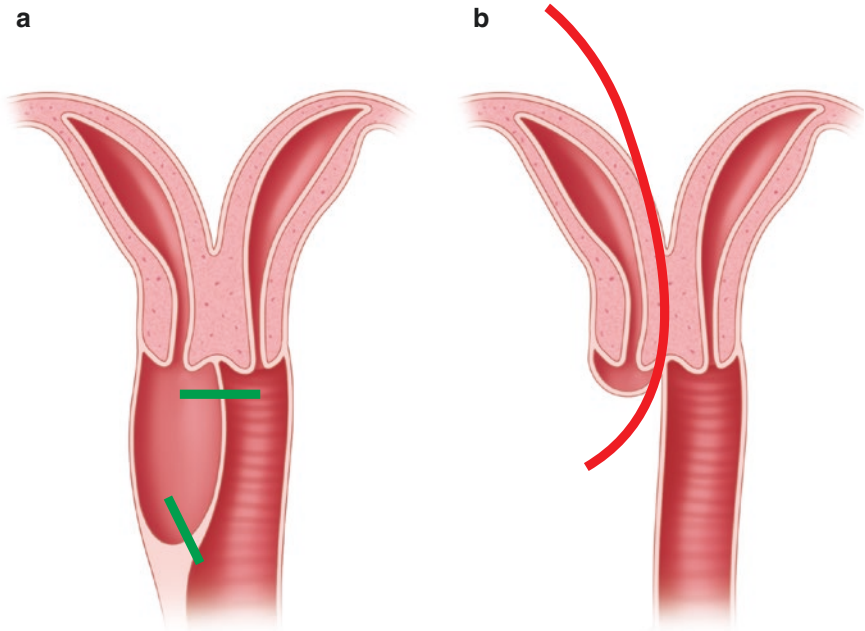
Fig. 6.3 (**a**) Large hematocolpos—better approach is vaginal wall resection. (**b**) Small hematocolpos, consider resection of uterine horn, cervix, and vagina, as connecting the hemivaginas carries high risk of stenosis

location of the hematocolpos. With gentle pressure on the abdomen, the hematocolpos will become more prominent. An 18 g needle can be placed through the vaginal wall into the hematocolpos to confirm proper location when menstrual blood is withdrawn by aspiration. An incision can then be made over the entry with the needle to get into the hematocolpos. This opening may be further enlarged using a crile or right-angle clamp. A suction may be placed through the opening into the hematocolpos to evacuate the fluid. Once the hematocolpos has been decompressed, the vaginal septum can be resected using either a bovie or other sealant/cutting device. The opening should be made as large as possible to decrease the possibility of restenosis. However, great care must be taken to avoid injuring the bladder and rectum, which are closer than one would think. Serial rectal exams can be very helpful. Filling the bladder in a retrograde fashion can help delineate the bladder. The vaginal opening may be marsupialized by over sewing with interrupted stitches using absorbable suture. The previously obstructed uterine horn has been demonstrated to carry a pregnancy 36.5% of the time so is valuable for future fertility. Vaginal delivery is possible. In those cases where the uterine anomaly is a complete septate uterus, removal of the uterine septum at the same time as one deals with the obstructed hemivagina is not ideal, as distention of the ipsilateral uterine cavity may lead to distortion of the uterine septum and difficulty with septum resection. The uterus should be allowed to return to its normal shape before uterine septum incision.

Hemi-hysterectomy with removal of the cervix and ipsilateral hemi-vagina is another approach that is typically reserved for those cases where the obstructed hemivagina is small or located far from the normal vagina, making marsupialization difficult and restenosis likely [10]. In these cases, the procedure may be performed by laparoscopy, robot-assisted laparoscopy, or laparotomy depending on the anatomy and surgeon skill set. It is important to remove the vaginal tissue at the time of hemi-hysterectomy. With arousal the vaginal tissue will secrete fluid, which may lead to accumulation of fluid in the hemi-vagina and cause pain and dyspareunia. In addition, the ipsilateral fallopian tube should be removed to decrease the risk of ectopic pregnancy.

Discussion

Double uterus with obstructed hemivagina is a well-recognized Mullerian anomaly associated with unilateral outflow obstruction and simultaneous regular menstrual cycles from the non-obstructed side. This anomaly is referred to as double uterus with obstructed hemivagina, or obstructed hemivagina and ipsilateral renal anomaly (OHVIRA), or Herlyn-Werner-Wunderlich syndrome (HWW). This anomaly typically presents shortly after puberty with severe unilateral dysmenorrhea. As many as 95% of individuals have associated ipsilateral renal agenesis, which may be detected during prenatal fetal ultrasonography and should be recognized as a risk factor for the anomaly. Delay in diagnosis is common and may be weeks to years in duration. This delay may be attributed in part to lack of recognition of obstructed Mullerian anomalies in the differential diagnosis. Treatment is surgical and should be planned with future fertility in mind. A vaginal approach with resection of vaginal septum and marsupialization to maintain patency between the hemivaginas is the preferred approach. In those rare cases where the obstructed hemivagina is small or not in proximity to the contralateral patent hemivagina, a hemihysterectomy with removal of cervix, hemivagina, and ipsilateral fallopian tube may be necessary. If the diagnosis is made before there is significant damage to the upper reproductive tract, fertility is normal, with 36.5% of pregnancies occurring in the previously obstructed hemiuterus.

References

1. Candiani GB, Fedele L, Candiani M. Double uterus, blind hemivagina, and ipsilateral renal agenesis: 36 cases and long-term follow-up. Obstet Gynecol. 1997;90(1):26–32.
2. Fedele L, Motta F, Frontino G, Restelli E, Bianchi S. Double uterus with obstructed hemivagina and ipsilateral renal agenesis: pelvic anatomic variants in 87 cases. Hum Reprod. 2013;28(6):1580–3.
3. Smith NA, Laufer MR. Obstructed hemivagina and ipsilateral renal anomaly (OHVIRA) syndrome: management and follow-up. Fertil Steril. 2007;87(4):918–22.

4. Pfeifer SM, Attaran M, Goldstein J, Lindheim S, Petrozza J, Rackow B, et al. ASRM Mullerian anomalies classification 2021. Fertil Steril. 2021;116(6):1238–52.
5. Troiano RN, McCarthy SM. Mullerian duct anomalies: imaging and clinical issues. Radiology. 2004;233(1):19–34.
6. Tong J, Zhu L, Lang J. Clinical characteristics of 70 patients with Herlyn-Werner-Wunderlich syndrome. Int J Gynaecol Obstet. 2013;121(2):173–5.
7. Grimbizis GF, Gordts S, Di Spiezio Sardo A, Brucker S, De Angelis C, Gergolet M, et al. The ESHRE/ESGE consensus on the classification of female genital tract congenital anomalies. Hum Reprod. 2013;28(8):2032–44.
8. Haddad B, Barranger E, Paniel BJ. Blind hemivagina: long-term follow-up and reproductive performance in 42 cases. Hum Reprod. 1999;14(8):1962–4.
9. Vercellini P, Daguati R, Somigliana E, Viganò P, Lanzani A, Fedele L. Asymmetric lateral distribution of obstructed hemivagina and renal agenesis in women with uterus didelphys: institutional case series and a systematic literature review. Fertil Steril. 2007;87(4):719–24.
10. Romanski PA, Bortoletto P, Pfeifer SM. Unilateral obstructed Mullerian anomalies: a series of unusual variants of known anomalies. J Pediatr Adolesc Gynecol. 2021;34(5):749–57.

Chapter 7
Dysmenorrhea

Hey-Joo Kang

Case

A 32-year-old nulligravida presents with increasing dysmenorrhea for the past 2 years. She has gone to the ER on multiple occasions for pain management especially during the first 2 days of menstruation, oftentimes missing work. She works as a nurse, and her husband is an ER physician. They have been married for 5 years and have been trying to conceive for 1 year without success. Menarche was at age 13 and initially irregular but became monthly at age 16. Menses were not painful in the beginning but became progressively painful at age 26. Her periods last 5–7 days and are not particularly heavy in flow.

Her past medical history is significant for migraines with aura, limiting her hormonal management with oral contraceptive pills. She has tried progesterone-only pills with a small degree of improvement. She has a history of anxiety/depression and has been on a low-dose SSRI for the past 2 years with some improvement. She has never had surgery. Her family history is notable for her mother having had a TAH/BSO in her 40s for chronic pelvic pain and heart disease in both her grandparents. She has a sister who was diagnosed with endometriosis and fibroid uterus but was able to conceive naturally after 6 months of attempting pregnancy. She is a nonsmoker with minimal alcohol consumption.

Physical exam showed normal vital signs and a BMI of 23 kg/m^2. Abdominal and rectal exams were normal, and stool guaiac test was negative. Pelvic exam showed normal female external genitalia and healthy-appearing vaginal mucosa and cervix with small black lesions dotted along the transformation zone. On bimanual exam, uterus was felt to be normal sized, but bilateral adnexa were slightly enlarged, although exam was limited due to patient discomfort. Transvaginal ultrasound

H.-J. Kang (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: hek9004@med.cornell.edu

showed a normal uterine size and shape, except for a small 1 cm intramural fibroid. There was no evidence of adenomyosis and a normal trilaminar endometrial stripe of 8 mm at midcycle was seen. Adnexa were remarkable for a 3 cm hemorrhagic cyst on the right ovary and a 2.5 cm similar appearing cyst on the left.

Laboratory evaluation showed a normal CBC. Her anti-Mullerian hormone level was 3.4 ng/mL.

She was counseled on options for treating her dysmenorrhea and bilateral hemorrhagic cysts compatible with endometriomas. Though they were interested in fertility, her pain bothered her more. She was treated initially with a Mirena IUD. Her menses became less painful, but she complained of persistent irregular bleeding and requested removal after 3 months. She ultimately chose surgical management with a laparoscopic bilateral cystectomy and fulguration of endometriotic implants. There was no bowel involvement, and bilateral tubes were patent. Postoperative course was entirely uneventful. Follow-up exam 3 months later showed complete resolution of pain, and an ultrasound revealed bilaterally normal-appearing ovaries. Repeat AMH was stable at 3.1 ng/mL, and she was able to conceive naturally 5 months later.

Discussion

Dysmenorrhea is painful menstruation occurring with regularity and is present in 30–50% of young, reproductive-aged women [1]. When severe, it can interfere with normal daily activities and lead to frequent absenteeism from work or school. Primary dysmenorrhea is the presence of lower abdominal pain during menses without an identifiable disease that attributes to the pain. It occurs more often in adolescent and younger women and is a diagnosis of exclusion, thus it requires a workup before a primary dysmenorrhea diagnosis can be made. It is often underdiagnosed and underreported. A detailed menstrual history is critical as primary dysmenorrhea often begins within 1 year of menarche. Secondary dysmenorrhea is more often found in women in their 30–40s and has identical symptoms of painful menstruation with demonstrable pathology, most commonly being benign conditions that likely develop throughout one's late 20s and become initially symptomatic in the 30s.

At the beginning of menses, endometrial sloughing releases prostaglandins that induce uterine contractions and cause pain [2]. When contractions are particularly strong, anaerobic metabolites accumulate when intrauterine pressure exceeds arterial pressure. In addition, stretch receptors are likely activated, additively contributing to the degree of pain. The timing of pain usually begins 1–2 days before the onset of menstrual bleeding and gradually lessens over 24–72 h. It is confined to the lower midline abdomen, but for some women the pain radiates to the back or upper thighs. When the pain is severe, gastrointestinal or neurologic symptoms such as nausea or headache often accompany the dysmenorrhea. Secondary causes of painful menstruation typically begin after age 25 and can be caused by common

conditions such as fibroids, endometriosis, and adenomyosis. Given that primary dysmenorrhea is a diagnosis of exclusion, some symptoms suggestive of secondary dysmenorrhea include onset of symptoms after age 25, abnormal uterine bleeding, i.e., heavy or irregular, lateralized pain, dyspareunia, dyschezia, and absence of nausea or headaches during menstruation. Approximately 40% of women with dysmenorrhea caused by endometriosis have physical findings on pelvic exam.

In younger women who are not sexually active, a pelvic exam is not needed when the history is highly suggestive of primary dysmenorrhea. The latter is usually not associated with any physical finding, abnormal laboratory or imaging studies, and the pelvic exam is typically normal. Tests that should be performed in sexually active individuals include cervical cultures for chlamydia/gonorrhea and a urine culture.

The only routine imaging needed to exclude a secondary cause for dysmenorrhea is a pelvic ultrasound if the clinical exam is normal, or in obese patients whose clinical exam is difficult. Diagnostic laparoscopy is rarely indicated and only if a secondary cause is highly suspected and when routine medical treatment fails to restore adequate quality of life.

When secondary dysmenorrhea is suspected, the differential diagnosis includes endometriosis, adenomyosis, fibroids, ovarian cysts, chronic PID/pelvic adhesions, cervical stenosis or Mullerian anomalies, and pelvic congestion syndrome. Non-gynecologic disorders include inflammatory bowel disease, irritable bowel syndrome, and psychogenic disorders.

For primary dysmenorrhea, treatment should be aimed at relief of pain and return to normal productivity. Patient education and reassurance—including the commonness of the diagnosis and low impact on future fertility—should be discussed. Heat packs and exercise are initial steps, followed by—or in combination with—NSAIDs and acetaminophen. NSAIDs inhibit prostaglandin synthesis and appear to be more effective in pain relief compared to acetaminophen [3]. The dose of NSAIDs should start at 400–600 mg every 6 h or 800 mg every 8 h with a maximum daily dose of 2400 mg/day starting with onset of menses or 24 h prior. For those at high risk of gastric ulcers, acetaminophen should be the first-line choice. Because prostaglandins play an important role in ovulation, those trying to conceive who require pain relief beyond the tenth day of their menstrual cycle should switch over from NSAIDs to acetaminophen after the seventh day.

If first-tier treatment with NSAIDs/acetaminophen is ineffective, then hormonal therapy is the next tier of treatment. Estrogen-progestin contraceptive agents maintain a thin endometrium, thus creating a lining with only small levels of arachidonic acid—the substrate for prostaglandin synthesis. This in turn reduces blood flow, contractility, and pain. The patient is first screened for contraindications of combination pill use such as migraines with aura, a personal history of blood clots, or uncontrolled hypertension [4]. Estrogen doses of >35 μg vs <35 μg show a similar degree of pain relief, as do continuous vs cyclic way of administration [5]. When treating for dysmenorrhea, pill regimens with shorter placebo duration or extended length formulations (3 months of active pills/placebo week) are ideal. Oral, transdermal, and vaginal rings are all acceptable options depending on the patient's

comfort and choice. There has been no evidence to show decreased efficacy of one route over another, although existing data is mostly based upon the traditional oral route.

For those who cannot take estrogen, a progesterone-only pill as an option should be discussed. Different preparation such as norethindrone 0.35 mg tablets, drospirenone 4 mg tablets, and norethindrone 5 mg tablets can be considered. Progestin-only pills are dispensed in familiar-appearing packs for those accustomed to combination oral contraceptive pills but have a higher incidence of irregular bleeding and do not inhibit ovulation as reliably as the combination pills. Injectable contraception (depot-Provera) has a 50% amenorrhea rate after 1 year of use, but there may be a delay to ovulation once discontinued. Levonorgestrel IUDs (20 μg) also reduce dysmenorrhea, and 20% of users have amenorrhea after 1 year. Implantable, single-rod etonogestrel-releasing options are also available, but less well studied for relief of dysmenorrhea. GnRH agonist treatment is another option but is typically reserved for the most refractory cases as it can increase the risk of osteopenia.

If hormonal treatment is insufficient in relieving dysmenorrhea, the next tier in treatment is offered. Diagnostic laparoscopy to assess and excise/fulgurate endometriosis lesions or diagnose pelvic congestion syndrome is the next reasonable step. If endometriosis is found, hormonal therapy should be continued to reduce risk of recurrence. If the patient has completed her childbearing or does not wish to have children, endometrial ablation is a reasonable option, especially for those whose dysmenorrhea is associated with menorrhagia. Transcutaneous electrical nerve stimulation (TENS) are 12 weekly treatments and can be used in conjunction with NSAIDs, hormonal contraception, and heat therapy. It is hypothesized to raise the threshold for pain signals or reduce perception of pain by sending high frequency of afferent pulses to the uterus [6].

Alternative medicine options such as acupuncture or acupressure can be included in discussions and can be used in refractory cases in conjunction with the first- or second-tier agents. Level A evidence is not supportive of alternative medicine as a primary treatment for dysmenorrhea. A variety of diets/dietary supplements have also been studied with some reduction in dysmenorrhea—vegetarianism, high dairy diet, increased vitamin D3, B6, and E—in small studies that have shown limited benefit in pain reduction [7].

The goal of treatment should be to educate the patient on potential causes of dysmenorrhea and to find the minimal effective dose of treatment needed to resume her normal daily activities. NSAIDs, acetaminophen, and heat therapy are the first-line therapy. The second-line treatments should include hormonal options, and the third-line options are more invasive laparoscopy/hysteroscopy and TENS. Regular follow-up visits are helpful to women with dysmenorrhea to review symptoms and monitor the degree of improvement with various treatments, ensuring the patient is receiving optimal care.

References

1. Klein JR, Litt IF. Epidemiology of adolescent dysmenorrhea. Pediatrics. 1981;68:661–4.
2. Dawood MY. Primary dysmenorrhea: advances in pathogenesis and management. Obstet Gynecol. 2006;108(2):428–41.
3. Armour M, Parry K, Al-Dabbas MA, et al. Self-care strategies and sources of knowledge on menstruation in 12,526 young women with dysmenorrhea: a systematic review and meta-analysis. PLoS One. 2019;14(7):e0220103.
4. Wong CL, Farquhar C, Roberts H, Proctor M. Oral contraceptive pill for primary dysmenorrhea. Cochrane Database Syst Rev. 2009;2009(4):CD002120.
5. Edelman AB, Gallo MF, Jensen JT, et al. Continuous or extended cycle vs cyclic use of combined oral contraceptives for contraception. Cochrane Database Syst Rev. 2014; 2014(3):CD004695.
6. Smith RP, Heltzel JA. Interrelation of analgesia and uterine activity in women with primary dysmenorrhea. A preliminary report. J Reprod Med. 1991;36(4):260–4.
7. Barnard ND, Scialli AR, Hurlock D, Bertron P. Diet and sex-hormone binding globulin, dysmenorrhea, and premenstrual symptoms. Obstet Gynecol. 2000;95(2):245–50.

Chapter 8
Abnormal Uterine Bleeding

Ashley Aluko and Joshua Stewart

Case

A 34-year-old nulliparous woman presents to the office with irregular periods. She has been on a combined oral contraceptive pill (COC) since college. She stopped the COC approximately 6 months prior to evaluation when she and her husband began trying to conceive. She reports that she was having a regular withdrawal bleed every month while on COC. She has had two menstrual cycles since stopping the COC. The first cycle was very heavy and lasted for approximately 8 days. The second cycle occurred approximately 90 days later, with lighter bleeding lasting for 4 days. She has been monitoring ovulation with urinary ovulation predictor kits but has become increasingly frustrated as they never turned positive.

She reports menarche at age 12 associated with normal pubertal development. Her periods were initially irregular, occurring every 30–60 days with variable amount of flow. Her cycles eventually normalized at age 14 years. Her medical history is significant for exercise-induced asthma for which she occasionally needs to use an albuterol inhaler, as well as anxiety which is well-controlled with psychotherapy. She has never had surgery. She has a sister with polycystic ovarian syndrome (PCOS), but otherwise denies any pertinent family history.

On physical exam, she is a well-appearing female with BMI 27 kg/m². There is no palpable mass on her thyroid. Pelvic exam reveals normal external female genitalia, normal cervix, and a normal-sized, mobile uterus with no adnexal mass. A transvaginal ultrasound reveals a normal uterus with thin endometrial stripe (3 mm) without evidence of polyps or myomas, and normal-appearing ovaries with an antral follicle count of 13.

A. Aluko · J. Stewart (✉)

Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: jds9013@med.cornell.edu

Urine and serum hCG were negative. A complete blood count (CBC) revealed mild iron-deficiency anemia. Thyroid-stimulating hormone (TSH) and prolactin were normal. A hormonal profile revealed the following on cycle day 47: estradiol 38 pg/mL, luteinizing hormone (LH) 4 IU/L, follicle-stimulating hormone (FSH) 5.1 IU/L, anti-Mullerian hormone 1.10 ng/mL.

A diagnosis of anovulatory cycles following long-term COC use was made. The patient was subsequently monitored over the following 3–4 months. Her cycle lengths became somewhat shorter; however, cycles still occurred every 40–50 days. As the couple was interested in conceiving, the patient began clomiphene citrate for ovulation induction. She ultimately conceived after three cycles of clomiphene citrate and timed intercourse and is now in her second trimester.

Discussion

Abnormal uterine bleeding (AUB) is defined as menstrual flow outside of the normal volume, duration, regularity, or frequency. The American College of Obstetricians and Gynecologists (ACOG) supports the adoption of the PALM-COEIN nomenclature system developed by the International Federation of Gynecology and Obstetrics (FIGO) in 2011, which divides the etiologies of AUB into structural and non-structural causes (Fig. 8.1).

The pathophysiology of AUB can be roughly divided into two categories: ovulatory AUB and AUB due to ovulatory dysfunction. With ovulatory AUB, the hypothalamic–pituitary–ovarian axis is intact and steroid hormone profiles are generally normal. Common mechanisms include abnormal prostaglandin synthesis and receptor upregulation [1], increased fibrinolytic activity [2], and increased tissue plasminogen activator activity [3]. In contrast, AUB due to ovulatory dysfunction is typically due to an endocrinopathy, such as polycystic ovary syndrome (PCOS), thyroid disease, hyperprolactinemia, and other mechanisms related to unopposed estrogen.

When the cause of AUB is being determined, we favor a differential diagnosis approach guided by the age of the patient. In reproductive-aged women as in our case example, the most common causes are pregnancy, anovulation, an endocrinopathy, structural causes (polyps, fibroids, adenomyosis), medications, infections, and malignancy. In premenarchal girls, special considerations should include a foreign body, trauma or abuse, sarcoma botryoides, and precocious puberty as differentials. In adolescence, anovulation due to hypothalamic immaturity is most common, but other special considerations may include hypothalamic stress (exercise-induced or an eating disorder) or coagulation disorders. In postmenopausal women, atrophy, malignancy, and medication effects should be suspected.

The evaluation of AUB starts with a detailed history, including the age of menarche (and menopause if applicable), the menstrual bleeding pattern (timing, frequency, last menstrual period, duration, quantity), associated symptoms (pain,
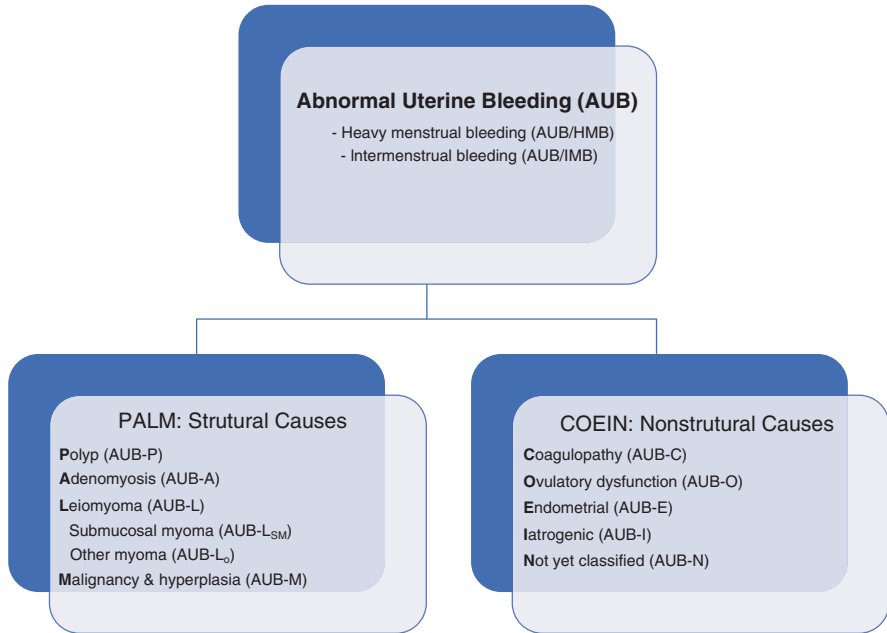
Fig. 8.1 AUB nomenclature. Source: Diagnosis of Abnormal Uterine Bleeding in Reproductive-Aged Women. Practice Bulletin No. 128. American College of Obstetricians and Gynecologists. July 2012 (Reaffirmed 2016)

vaginal discharge, fever), screening for signs and symptoms of possible hemostatic disorder, as well as pertinent medical, surgical, medication, and family histories [4]. Physical exam should pay special attention to the presence or absence of ecchymosis, thyromegaly, clinical signs of hyperandrogenism (hirsutism, acne, male pattern balding), or acanthosis nigricans. Laboratory testing should include a pregnancy test (blood and/or urine), as well as a CBC, TSH, prolactin, and targeted screening for bleeding disorders. When appropriate, a Pap smear and cultures for Chlamydia trachomatis/Gonorrhea should be obtained. In post-pubertal women, estradiol, luteinizing hormone (LH), and progesterone levels can help determine ovulatory status. Transvaginal ultrasonography is the primary imaging modality utilized in the evaluation of AUB in reproductive age and post-menopausal women. Saline infusion sonohysterography (SIS), magnetic resonance imaging (MRI), and hysteroscopy may also be beneficial in cases of structural lesions. Endometrial sampling should be performed in women with unopposed estrogen exposure, as well as those with risk factors for endometrial hyperplasia—obesity, chronic anovulation (PCOS), history of breast cancer, SERM (tamoxifen) use, family history of endometrial, ovarian, breast, or colon cancer and/or thick endometrial lining on ultrasound.

Treatment options depend upon the etiology of the AUB and the patient's overall goals, namely whether the patient is attempting to conceive in the future (either in the near or long term). When AUB is thought to be due to an endocrinopathy or

bleeding disorder, appropriate medical management is indicated. In cases of anovulatory bleeding or unopposed estrogen, medical therapy is often very effective. Medical treatment options may include conjugated equine estrogen (acute), combined oral contraceptives, a cyclic progestin (e.g., medroxyprogesterone acetate), tranexamic acid (acute or chronic), levonorgestrel intrauterine device, or gonadotropin releasing hormone agonist (leuprolide). In cases of failed medical therapy or known surgical indication (structural causes), hysteroscopy/D&C, endometrial ablation, or hysterectomy (definitive treatment) may be indicated. In cases of atypical hyperplasia or malignancy, we would refer to a gynecologic oncologist for further evaluation and definitive treatment.

In conclusion, AUB is an extremely common reason for women to present for gynecologic evaluation. We recommend a diagnostic and treatment approach guided by the age of the patient and her overall goals.

References

1. Smith SK, Abel MH, Kelly RW, Baird DT. Prostaglandin synthesis in the endometrium of women with ovular dysfunctional uterine bleeding. Br J Obstet Gynaecol. 1981;88(4):434–42.
2. Dockeray CJ, Sheppard BL, Daly L, Bonnar J. The fibrinolytic enzyme system in normal menstruation and excessive uterine bleeding and the effect of tranexamic acid. Eur J Obstet Gynecol Reprod Biol. 1987;24(4):309–18.
3. Gleeson N, Devitt M, Sheppard BL, Bonnar J. Endometrial fibrinolytic enzymes in women with normal menstruation and dysfunctional uterine bleeding. Br J Obstet Gynaecol. 1993;100(8):768–71.
4. Diagnosis of abnormal uterine bleeding in reproductive-aged women. Practice Bulletin No. 128. American College of Obstetricians and Gynecologists. July 2012 (Reaffirmed 2016).

Chapter 9
Hirsutism

Check for updates

Nina Vyas and Joshua Stewart

Case

A 51-year-old multiparous woman presents with progressive hair growth on her face and lower abdomen. She reports menarche at age 11 and she was diagnosed with polycystic ovarian syndrome (PCOS) in her teens. Her diagnosis at that time was presumably based on elevated serum testosterone concentrations and irregular menses. For most of her reproductive years, she was placed on oral contraceptive pills and used depilatories to remove unwanted hair growth. In her early 30s, she successfully conceived twice with the use of clomiphene citrate for ovulation induction and has had two healthy children. Recently, she visited her gynecologist as she believed she was in menopause after being amenorrheic for over 12 months with climacteric symptoms of vaginal dryness and hot flashes. She also told her gynecologist that during these past 12 months, she has noticed a gradual increase of thick and dark hairs on her chin, lower abdomen, and upper inner thighs as well as a receding hair line. Her husband has noticed a deepening in her voice. Her gynecologist referred her to a reproductive endocrinologist for further evaluation.

In addition to the diagnosis of PCOS, her medical history is significant for insulin-dependent type 2 diabetes mellitus, anxiety, and depression. She has had two prior cesarean deliveries, and no other surgical history. Her medications include nightly and meal-time insulin as well as oral metformin. She has no allergies to any medications. Her family history is significant for a history of PCOS in her mother and hypertension in her father. There is no family history of cancer. She has a remote history of tobacco use, denies illicit drug use, and consumed alcohol occasionally.

On physical exam, she had normal vital signs and a BMI of 31.4 kg/m^2. On inspection, she had dark hairs on her chin and lower abdomen, with a

N. Vyas · J. Stewart (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: jds9013@med.cornell.edu

Ferriman-Gallwey score of 20. Additionally, she had active acne on her face and upper back. There was no evidence of acanthosis nigricans. An external genital exam revealed an enlarged clitoral hood with a length of 11 mm and dark thick hairs along the upper inner thigh. She had mild thinning of the vaginal mucosa and a normal-appearing cervix. Bilateral ovaries were palpable on bimanual exam. A transvaginal ultrasound revealed a thickened endometrial stripe to 12 mm and enlarged bilateral ovaries measuring a volume greater than 10 cm^3 each. There were two antral follicles noted on the right ovary and one antral follicle on the left, with no discrete ovarian masses visualized.

Urine and serum pregnancy tests were negative. Serum testing was notable for a total testosterone of 213 ng/dL, DHEA-S of 101 μg/dL, FSH of 47.3 mIU/mL, and LH of 45.2 mIU/mL. TSH was 1.8 mIU/L, and prolactin was 13 ng/mL. HgbA1C was 8.3%. An adrenal CT was unremarkable. Given her thickened postmenopausal endometrial lining, an endometrial biopsy was performed, which revealed secretory endometrium and no evidence of hyperplasia.

The leading diagnosis at this time was bilateral ovarian stromal hyperthecosis. The patient was counseled regarding treatment options, including bilateral oophorectomy versus long-term GnRH agonist therapy. She opted to undergo laparoscopic bilateral salpingo-oophorectomy, and pathology confirmed the diagnosis of ovarian stromal hyperthecosis.

Over the 12 months following her surgery, her serum testosterone levels normalized. Additionally, her acne improved significantly, and the amount of hair growth on her chin and lower abdomen improved slightly but did not fully resolve. The deepening of her voice and clitoral enlargement had not improved over the course of the year. She began a diet and exercise program, and her BMI improved to 25.6. She continued her insulin therapy, albeit her insulin requirement had significantly reduced with an improved HgbA1C to 6.3%.

Discussion

Hirsutism is defined as the irreversible growth of terminal hairs in androgen-dependent areas, usually caused by increased circulating androgens. These areas include, but are not limited to, the upper lip, chin, mid-sternum, buttocks, upper inner thigh, and upper and lower back and abdomen.

In utero, hair follicles are developed between 8 and 10 weeks of gestation, reaching approximately five million hair follicles by the 22nd week of gestation. There are two categories of hairs, vellus and terminal. Vellus hairs are soft, fine, and non-pigmented, while terminal hairs are long, coarse, and pigmented. The number of hair follicles does not increase or decrease over an individual's lifetime; however, the follicle size and type of hair can change in response to androgens and environmental stimuli [1].

The three phases of hair growth are: the growth phase, the involution phase, and the resting phase, namely anagen, catagen, and telogen, respectively. Anagen, or the

growth phase, varies by the location of the body hair. For example, facial hair's growth phase is approximately 4 months, while scalp hair's is 2–5 years. Catagen, or the involution phase, lasts 2–3 weeks, marking the end of hair growth while the outer root sheath shrinks and attaches to the root of the hair. Telogen, or the resting phase, refers to the time spent before the hair is released by the follicle. Of note, this phase generally lasts 3–4 months [1].

Hirsutism can occur in both reproductive-aged and postmenopausal women, although it is more common in reproductive-aged women. The Ferriman-Gallwey (F-G) score is the most commonly used grading tool to quantify hair growth, rating nine androgen-sensitive sites from 0 to 4 [2]. A score of 8–15 indicates mild hirsutism, 16–25 indicates moderate hirsutism, while >25 reflects severe hirsutism. There is a fluctuation between ethnic groups, with some groups having a higher or lower threshold for what is considered "normal" body hair, and clinicians should be aware of these ethnic variations [3].

Androgen stimulation of hair follicles requires the conversion of testosterone to dihydrotestosterone (DHT). This exposure can increase hair follicle size, hair fiber diameter, and the proportion of time hairs spend in the anagen phase, allowing them to grow in length. The sensitivity of hair follicles to androgens is determined, in part, by the local level of 5α-reductase activity in the vicinity of the pilosebaceous unit, the functional unit of each hair follicle. This androgen exposure is the trigger for a vellus hair to become a terminal hair, leading to hair growth in the sex-dependent areas listed above [4].

The approach to a woman with hirsutism is multi-fold. In taking a detailed history, it is important to understand if the hair growth was gradual or rapid in onset. Thorough medical, medication, and hair-removal treatment histories are needed to understand the etiology and extent of the hair growth. Physical exam can show hair growth, acne, signs of acanthosis nigricans, or clitoral enlargement. Imaging is vital, as transvaginal US can help to evaluate ovarian volume and antral follicle count, as well as detect ovarian masses, while adrenal CT is used to discover androgen-secreting tumors. Some ovarian androgen-secreting tumors are not always large enough to detect on ultrasonography or MRI, and in these cases, ovarian and adrenal vein sampling can be done to localize the source and laterality of elevated androgen concentrations. This is more commonly performed in reproductive-aged women versus postmenopausal women to avoid unnecessary removal of an ovary.

Causes of hirsutism can be physiologic, idiopathic, iatrogenic, or pathologic. Physiologic changes are likely due to normal ethnic variations in hair growth pattern. Women with "idiopathic hirsutism" have normal serum androgen levels and normal menses, but there is elevated 5α-reductase activity converting testosterone to the more potent DHT causing androgenic effects locally at the hair follicle [4]. Iatrogenic causes include use of androgenic steroids or medications such as minoxidil or valproate. Pathologic etiologies include PCOS (covered in a separate chapter), classical and non-classical congenital adrenal hyperplasia, pregnancy luteomas, theca lutein cysts, Hyperandrogenism Insulin Resistance and Acanthosis Nigricans syndrome (HAIR-AN), Cushing's syndrome, hyperprolactinemia, and androgen-secreting tumors of the ovary and adrenal glands. Although these androgen-secreting

tumors account for only 0.2% of women with hirsutism, they are the most serious cause, with 50% being malignant at the time of diagnosis [5].

In the postmenopausal woman, new onset hyperandrogenism is rare. The most likely causes are ovarian stromal hyperthecosis or androgen-secreting tumors. Hyperandrogenism in the postmenopausal woman generally manifests as hirsutism and alopecia. Additionally, some women may experience postmenopausal bleeding due to the peripheral conversion of high levels of circulating testosterone to estradiol. In the setting of a thickened endometrial stripe or postmenopausal bleeding, an endometrial biopsy is recommended to rule out underlying hyperplasia.

In women presenting with hirsutism, we recommend serum biochemical testing, including total testosterone, hCG, TSH, an early morning 17-hydroxyprogesterone, prolactin, and FSH. Of the androgens secreted in women, testosterone and DHT have the most androgenic impact on hair follicles. Women with PCOS generally have total testosterone levels above 45 ng/dL but below 150 ng/dL. Levels over 150 ng/dL are correlated with virilization. Virilization can manifest as hirsutism, acne, alopecia, deepening of the voice, and/or clitoromegaly. Clitoromegaly is diagnosed based on clitoral length of the glans or the clitoral index (length >10 mm or an index of >35 mm^2) [6]. The total testosterone concentration does not generally correlate with the extent of virilization, as total testosterone also includes the testosterone bound to sex hormone-binding globulin (SHBG), roughly 80%. We do not recommend routinely evaluating serum DHEA-S, as mild elevations are unlikely to affect management. However, if there is concern for an androgen-secreting tumor, DHEA-S should be checked, and any levels above 700 µg/dL require further evaluation to rule out an adrenal tumor.

The approach to management of hyperandrogenism in the reproductive-aged woman includes initiation of combined estrogen-progestin oral contraceptives (COCs). COCs act by decreasing gonadotropin secretion and increasing SHBG, which in turn decreases the amount of circulating free testosterone. Given the duration of the hair growth cycle, up to 6 months is generally needed to notice a change in hair growth or decrease in acne before making a dose adjustment or adding additional therapy. For those who fail monotherapy with COCs or have a contraindication to COCs, an antiandrogen, such as spironolactone, may be added. In women who have suspected hyperandrogenism and are currently on COCs, it is recommended to wait 8–12 weeks prior to testing serum androgen levels. However, if hirsutism is progressing while taking COCs, one must promptly look for an androgen-secreting tumor [6].

The patient in our vignette had pathology confirming a diagnosis of ovarian stromal hyperthecosis, a condition seen most commonly in postmenopausal women and thought to be caused by the proliferation and luteinization of theca cells, due to baseline elevated FSH and LH. The condition is generally characterized by total testosterone >150 ng/dL, gradual onset of virilization, and no evidence of ovarian tumor or adrenal tumor on imaging. Additionally, most women with ovarian stromal hyperthecosis have a history of PCOS in their reproductive years. On ultrasound, the ovaries appear larger than 10 cm^3, but unlike PCOS, very few follicles are seen

in the enlarged ovaries and the ovaries appear solid. Doppler US would most likely not reveal additional vascularity as would be seen with ovarian tumors.

The treatment of ovarian stromal hyperthecosis includes oophorectomy, either unilateral or bilateral depending on the laterality of the disease. As previously stated, in the reproductive-aged women, ovarian vein serum testing can differentiate the laterality, while in postmenopausal women, bilateral oophorectomy is recommended. The option to use GnRH agonist treatment to decrease LH and FSH can be considered in poor surgical candidates or those who choose to avoid surgery; however, this has been proven to be less efficacious at resolving hyperandrogenism [7].

After treating and correcting the hyperandrogenism, terminal hairs do not reliably revert to vellus hairs. However, other signs of virilization may partially reverse such as acne, clitoromegaly, and voice deepening. Obesity is often associated with many of the etiologies of hirsutism, which emphasize the importance of weight loss and a healthy diet to reduce peripheral aromatization leading to increased risk of endometrial hyperplasia.

In conclusion, when approaching a reproductive-aged or postmenopausal woman with hirsutism, it is important to perform a thorough history and physical exam, in addition to ordering the correct biochemical serum testing and imaging to elucidate the diagnosis. Effective therapy must be directed at the underlying etiology. This can range from using COCs for the functional etiology, to corticoid therapy, or surgery to remove an androgen-secreting tumor.

References

1. Paus R, Cotsarelis G. The biology of hair follicles. N Engl J Med. 1999;341(7):491–7.
2. Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. J Clin Endocrinol Metab. 1961;21:1440–7.
3. DeUgarte CM, Woods KS, Bartolucci AA, Azziz R. Degree of facial and body terminal hair growth in unselected black and white women: toward a populational definition of hirsutism. J Clin Endocrinol Metab. 2006;91(4):1345–50.
4. Taylor HS, Pal L, Seli E, Fritz MA. Speroff's clinical gynecologic endocrinology and infertility. 9th ed. Philadelphia, PA: Wolters Kluwer; 2020.
5. Cordera F, Grant C, van Heerden J, Thompson G, Young W. Androgen-secreting adrenal tumors. Surgery. 2003;134(6):874–80; discussion 880.
6. Martin KA, et al. Evaluation and treatment of hirsutism in premenopausal women: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2018;103:1233. https://pubmed.ncbi.nlm.nih.gov/29522147/. Accessed 11 Aug 2021.
7. Vollaard ES, van Beek AP, Verburg FAJ, Roos A, Land JA. Gonadotropin-releasing hormone agonist treatment in postmenopausal women with hyperandrogenism of ovarian origin. J Clin Endocrinol Metab. 2011;96(5):1197–201.

Chapter 10
Polycystic Ovarian Syndrome (PCOS)

Isaac Kligman

Case

A 28-year-old woman presents with increased hair growth, weight gain, and oligo-menorrhea. After menarche at age 10, she experienced irregular menstrual cycles until she was placed on oral contraceptive pills (OCP) at age 15. She then had monthly withdrawal bleeding predictably. She is recently married and plans to conceive within the next year. Therefore, OCP was stopped about 1 year ago. Since then, she has had only four menstrual periods. More specifically, menstrual cycles occurred irregular at once every 60–90 days; flow was heavy and lasted for 10–12 days. Her last menstrual period was 2 months prior to her visit. She denies having dysmenorrhea, dyspareunia, or history of sexually transmitted infections. Her PAP smears have always been normal. Her medical and surgical history were otherwise not contributory except for an allergy to penicillin, and she is currently taking Alprazolam for depression. Her family history was significant for endometrial cancer in her eldest sister when she was 42. She does not smoke, use alcohol, or any other recreational drugs.

On physical exam, vital signs were within normal limits, and she appeared to be comfortable. She weighed 220 lb at a height of 5 ft 6 in. (BMI: 35 kg/m^2). Neck appeared supple without any mass. Hair growth over upper lip was noted. Breast exam did not reveal any mass or nipple discharge. Abdomen was soft with a pannus and some striae; her waist circumference was 36 in. Of note, there were raised and velvety lesions around the fold of her neck and axillae. Pelvic exam was unremarkable, and transvaginal ultrasound revealed an 18 mm tri-laminar endometrial stripe and multiple small follicles aligned in the periphery of both ovaries; no dominant follicle was visualized. Laboratory results included an FSH level of 3.5 mIU/mL,

I. Kligman (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: ikligman@med.cornell.edu

LH level of 12 mIU/mL, E2 level of 125 pg/mL, P4 of level of 0.6 ng/mL, and an AMH of 10.8 ng/mL. Thyroid function tests and prolactin were within normal limits. Androgen panel revealed a slightly elevated total testosterone level, but DHEAS and 17 OHP levels were normal. Hemoglobin A1C level was 6.0%. Morning cortisol levels and lipid profile were normal. Urine pregnancy test was negative at the time of her visit.

The patient was prescribed medroxyprogesterone 10 mg daily for 10 days; a repeat ultrasound after her withdrawal bleed revealed an endometrial stripe of 3 mm. She was referred to a nutritionist and lost 25 lb over the following 4–6 months on a hypocaloric diet. She was also referred to a medical endocrinologist who started her on metformin 850 mg twice a day. With this treatment, her HgbA1C decreased to 5.6%. The patient resumed regular cycles, and 8 months later she achieved a viable pregnancy successfully.

Discussion

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. Its diagnosis, according to the Rotterdam criteria, relies on the presence of two of the three features: oligo- or anovulation, hyperandrogenemia/hirsutism, and polycystic appearance of ovaries on ultrasound.

Clinical presentation of PCOS varies—69% of affected women present with hirsutism, 74% with infertility, 51% with amenorrhea, 41–80% with obesity, and 21% with dysfunctional uterine bleeding. Women affected with this disorder have an increased risk of other morbidities such as insulin resistance, type II diabetes, cardiovascular disease, metabolic syndrome, non-alcoholic fatty liver disease, endometrial cancer, and depression. The prevalence of this syndrome ranges from 6% to 20% of the population, depending on geographical regions [1].

PCOS is a life-long disorder; many genetic factors have been implicated and progress is being made in identifying specific genetic determinants of the syndrome. For instance, the insulin receptor gene (INSR) has been validated as a candidate receptor gene in genome-wide amplification studies (GWAS). The pathophysiology of PCOS is complex, and the syndrome can be explained by hypothalamic–pituitary axis abnormalities that include abnormal hypothalamic secretion of GnRH, which in turn increases pituitary LH secretion and androgen production by the ovaries. An enzymatic defect involved with ovarian steroidogenesis could also be responsible for the increase in ovarian androgen production. Insulin resistance may also play a pivotal role in this syndrome through direct insulin action on pituitary and ovarian receptors, a phenomenon associated with metabolic consequences. Insulin resistance has a direct association with glucose intolerance, hypertension, vascular disease, dyslipidemia, and obesity [2–4].

Obesity plays an important role in the perpetuation of the syndrome and can exacerbate the clinical manifestations. It increases the risk of infertility, as it is associated with a high incidence of anovulation, as well as failure or delayed response

of ovulation induction with clomiphene citrate or gonadotropins. It has been reported to be associated with pregnancy loss and late pregnancy complications such as gestational diabetes and preeclampsia. However, many of these issues resolve completely after weight loss and bariatric surgery [5].

The differential diagnosis of this condition, which have to be ruled out, include other causes of ovulatory dysfunction and hirsutism such as androgen-producing tumors, congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia, thyroid dysfunction, hypothalamic dysfunction (hypothalamic amenorrhea), hyper-insulinemia, and the HAIR-AN syndrome (Hyperandrogenism, insulin resistance, and acanthosis nigricans).

One of the most important steps in the evaluation of this patient is obtaining a full history of the onset, progression, and duration of symptoms, as well as obtaining a careful menstrual history, medication intake, and the use of exogenous androgens.

Physical examination should include blood pressure measurement, weight, and BMI. A BMI between 25 and 30 is categorized as being overweight, while >30 is defined as obesity. General body inspection should follow to diagnose the presence of hirsutism, acne, alopecia, clitoromegaly, or acanthosis nigricans, a skin condition characterized by velvety, hyper-pigmented areas in the skin of the back of the neck, axillae, and underneath the breasts. The presence of acanthosis nigricans is usually considered as a marker for insulin resistance and signals the need for further evaluation. Waist circumference ≥35 in. is one of the components of metabolic syndrome. Pelvic examination may occasionally reveal bilaterally enlarged ovaries and should be confirmed by transvaginal ultrasound. A typical appearance of polycystic ovaries entails ≥12 follicles measuring 2–9 mm in diameter in each ovary, or an increase in ovarian volume (>10 cm^3). Moreover, ultrasound examination should include a thorough evaluation of the endometrium as the initial step to rule out the possible presence of endometrial hyperplasia or other endometrial abnormalities.

Laboratory testing should encompass the evaluation of hyperandrogenemia by obtaining total and free testosterone levels along with sex hormone binding globulin (SHBG). DHEA-S levels will help rule out adrenal causes of hyperandrogenemia, and elevated levels may point to an adrenal source. Other causes of ovulatory dysfunction and hirsutism should also be ruled out by measuring a morning level of 17-hydroxyprogesterone, with the purpose of excluding non-classical congenital adrenal hyperplasia due to 21 hydroxylase deficiency, especially in patients from populations at risk of the disease such as Ashkenazy Jews. An elevated basal level should prompt a follow-up with an ACTH stimulation test. FSH and LH levels should also be obtained since up to 60% of women with PCOS have a high LH/FSH ratio. Prolactin and TSH levels should also be evaluated since hyperprolactinemia is a known etiologic factor in oligo-amenorrhea, and hypothyroidism may be associated with hirsutism since abnormal thyroid function may in turn affect testosterone levels by altering circulating SHBG levels. Screening for Cushing syndrome should be performed only if there are any clinical features such as moon facies or abdominal striae. Evaluation of metabolic abnormalities should include an evaluation of hemoglobin A1C or a 2-h glucose tolerance test after the administration of a 75 g glucose load. Measurements of glucose and insulin will aid in the diagnosis of

insulin resistance with abnormal compensatory hyperinsulinemia being a key component of the pathophysiology of PCOS. A fasting lipid profile should also be obtained since women with PCOS are prone to have dyslipidemia as a component of the metabolic syndrome. Elevated AMH levels have been associated with PCOS although it is not currently used in establishing the diagnosis of PCOS [6].

Treatment of PCOS involves different approaches, but the mainstay of therapy involves lifestyle modification and weight loss to begin with. Pharmacologic treatment aims at ameliorating the effects of hyperandrogenemia, managing ovulatory dysfunction, and counteracting the effect of unopposed estrogen on the endometrium. It is well known that these patients exhibit a higher incidence of endometrial hyperplasia and even endometrial cancer, especially when they are anovulatory. Therefore, patients will benefit from reversing anovulation with ovulation-inducing agents.

Metformin (Glucophage®) is an oral agent that enhances glucose uptake by peripheral tissues decreasing hepatic glucose production and reducing hyperinsulinemia. This medication is usually prescribed in doses ranging from 500 mg to 2 g daily. Some studies have suggested improvement of ovulatory function with this regimen, but others have not found a benefit compared to ovulation induction agents such as clomiphene citrate. The above notwithstanding, metformin should still be considered a first-line treatment in obese women with PCOS due to its metabolic benefits [7]. Combined oral contraceptives (OCs) should be considered as the first line of treatment for women who do not intend to become pregnant. OCs carry the immediate benefit of increasing SHBG by the liver and decreasing circulating androgen levels; additionally, they provide progestational support of the endometrium, decreasing the incidence of endometrial hyperplasia. Low-dose OCs are recommended due to the potential dose-dependent effect on insulin sensitivity and potential metabolic risk factors. Antiandrogens, specifically spironolactone (Aldactone®), is an androgen receptor antagonist that can be administered concomitantly with OCs. Cyclic progestins alone (Provera®) can be used as an alternative to OCs in anovulatory women with PCOS who are not intending to achieve pregnancy to ameliorate the risk of endometrial hyperplasia. Progestins can also be administered parentally in depot preparation or in progestin-containing intrauterine devices. The effects of these preparations on metabolic risk factors have not been clearly elucidated.

The first-line treatment for women with PCOS diagnosed with anovulatory infertility has traditionally been clomiphene citrate which is an antiestrogenic agent that blocks hypothalamic estrogen receptors, in turn increasing GnRH secretion by the hypothalamus along with gonadotropin secretion by the pituitary, ultimately inducing ovulation. Clomiphene citrate increases FSH levels by 30–40% by day 3 or 4 after initiation of treatment. Most patients respond to a 50 or 100 mg dose daily for 5 days; the maximum effective dose is 150 mg. If there is no response to the 150 mg dose, the patient should be offered a different regimen for ovulation induction. About 75–80% of PCOS patients will ovulate with the oral regimen, with a conception rate of up to 22% per cycle (<35 years old in the absence of other infertility factors) and a 10% risk of multiple pregnancy. Aromatase inhibitors such as

letrozole (Femara®) have been used off label and recommended as primary or secondary treatment for anovulatory women with PCOS. This drug reduces circulating estrogen inhibiting the negative feedback at the hypothalamic-pituitary axis; this results in increased FSH secretion with resulting follicular development. Ovulation rates have been reported to be between 60% and 84%, with conception rates between 10% and 40% cumulatively. A meta-analysis has shown comparative pregnancy rates between women treated with clomiphene citrate and those treated with letrozole. More recently, however, a randomized controlled trial reported higher ovulation and live birth rates with letrozole. In patients that fail to ovulate with oral ovulation induction agents, injectable gonadotropins can be utilized [8]. Careful dosing is mandatory since the use of these agents is associated with development of a higher number of follicles, and therefore risks of multiple pregnancy and ovarian hyperstimulation syndrome (OHSS) have to be watched carefully. The latter is an iatrogenic condition associated with increased vascular permeability, third spacing with resultant ascites and hydrothorax with pulmonary edema, thromboembolic events, and kidney failure. It can be life-threatening in the most extreme cases. The regimen, when gonadotropins are used to induce ovulation in PCOS patients, is the so-called step-up protocol designed to reach a threshold serum concentration of FSH to achieve mono-follicular development. Gonadotropins are usually started on cycle day 2 of a spontaneous or an induced menses with doses as low as 37.5 IU daily. It is gradually increased depending upon the ovarian response determined by frequent ovarian ultrasound and estrogen level measurement. Despite a low-dose approach and careful monitoring, higher than expected response can still be encountered. If there is multi-follicular development (>3 dominant follicles) and/or estrogen levels >1000 pg/mL, the patient should be counseled regarding risks of OHSS and multiple pregnancy. Such cycle should be canceled, and the patient is advised against attempting to conceive during that cycle. An alternative to cancelation is to convert the treatment to in vitro fertilization (IVF). However, the latter approach may not be most desirable because the patient is not mentally prepared for IVF and cost may present as an issue. Moreover, while the number of follicles is too many for timed intercourse or intrauterine insemination (IUI), it may be low for IVF.

IVF should be considered in PCOS patients when oral agents or a low-dose gonadotropin approach either fails to induce ovulation or achieve a conception with timed intercourse or IUI, or the response is too high for pregnancy to occur safely as described above. The advantage of IVF compared to ovulation induction is that regardless of the number of follicles recruited or eggs retrieved, a single embryo will be transferred back to the patient, much reducing the risk of multiple pregnancies. Rest of the viable embryos can be frozen for future use. Moreover, in IVF, gonadotropins will be used together with a GnRH antagonist which, on one hand, is employed to prevent ovulation from occurring before the intended oocyte retrieval and, on the other hand, allows the utilization of a GnRH agonist, instead of hCG, to trigger ovulation which will significantly reduce the risk of OHSS. If risks of OHSS are still considered high, all embryos should be frozen to avoid pregnancy in that cycle. Patients can return for a frozen embryo transfer in a later cycle when OHSS risk is not present. A recent

multicenter trial that included more than 1500 patients concluded that women with PCOS who were at risk of OHSS and underwent a frozen embryo transfer in a subsequent cycle had a higher live birth rate and lower miscarriage rate than their counterparts who underwent a fresh embryo transfer during the stimulated cycle. Therefore patients should be aware that freezing the embryos up front will not impact negatively on their success rates.

Laparoscopic ovarian drilling, though not commonly practiced anymore, aims at decreasing the mass of androgen-producing thecal cells in the ovary. It can be performed with monopolar cautery or with laser cauterization. Reported ovulation rates range from 50% to 90% with conception rates of 70% cumulatively in young patients. However, studies available to date fail to show a benefit of ovarian drilling over conventional ovarian stimulation or IVF. Although patients are encouraged to conceive on their own after drilling, thus avoiding the risk of OHSS or multiple pregnancy, the risks of having a procedure under general anesthesia, adhesion formation around the ovaries and fallopian tubes, and potentially decreasing ovarian reserve are major drawbacks of this approach.

In conclusion, PCOS is a common endocrinopathy that has the potential to cause substantial metabolic and gynecologic sequelae. Anovulation is the main component of the syndrome responsible for many of its presentations including infertility. Addressing obesity and insulin resistance are extremely important in the management of these patients, and the treatment cascade should always start with diet and weight loss. Clomiphene citrate and letrozole, both oral agents remain as the first step in treating fertility-associated anovulation. If this fails, a very careful, low-dose gonadotropin stimulation with or without IUI should be next step. IVF will be the last resort for reasons illustrated above. There is insufficient data to recommend laparoscopic ovarian drilling over other conventional therapies. Considering that PCOS is a life-long syndrome, a multidisciplinary approach is necessary to take care of these patients at different stages of their lives.

References

1. Azziz R. Polycystic ovary syndrome. Obstet Gynecol. 2018;132:321–36.
2. American College of Obstetrics & Gynecology. Polycystic ovary syndrome. Practice Bulletin No. 194. Obstet Gynecol. 2018;131:e157–71.
3. Zimmerman LD, Setton R, Pereira N, Rosenwaks Z. Contemporary management of polycystic ovarian syndrome. Clin Obstet Gynecol. 2019;62(2):271–81.
4. Baskind NE, Balen AH. Hypothalamic-pituitary, ovarian and adrenal contributions to polycystic ovary syndrome. Best Pract Res Clin Obstet Gynecol. 2016;37:80–97.
5. Fauser BCJM, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, 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.
6. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome. A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute;

American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640–5.

7. Practice Committee of the American Society for Reproductive Medicine. Role of metformin for ovulation induction in infertile patients with polycystic ovary syndrome (PCOS): a guideline. Fertil Steril. 2017;108:426–41.

8. Balen AH, Morley LC, Misso M, Franks S, Legro RS, Wijeyaratne CN, et al. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. Hum Reprod Update. 2016;22(6):687–708.

Chapter 11
Hyperprolactinemia

Allison Petrini and Pak H. Chung

Case

A 27-year-old nulligravida with a 1-year history of primary infertility presented with 7 weeks of secondary amenorrhea. She reported menarche at age 12 and previously regular menses with 30-day cycles. Urine pregnancy tests at home were repeatedly negative. Upon questioning, she also revealed that for the past month, she has noticed cloudy white discharge from her breasts bilaterally. The discharge was not bothersome to her; however, in the setting of her amenorrhea, she has become increasingly concerned. She denied any visual disturbance, headaches, heat or cold intolerance, weight change, fatigue, constipation, or diarrhea.

She has been married for 2 years and attempting to conceive for the past year. She was up to date on her annual pap smears and breast examination which have been within normal limits. Her past medical history was significant for anxiety and depression. She was just initiated on escitalopram 3 weeks ago but did not take any other medications. She had a surgical history significant for a ruptured appendix at age 13 for which she had a laparoscopic appendectomy. Her family history was significant for type II NIDDM in her mother and coronary artery disease in her father. She did not have any family history of cancer. She was a nonsmoker, denied illicit drug use, and consumed alcohol minimally. Three months ago, when seen by her gynecologist, she was referred for a work-up of infertility but in the interim developed these symptoms of amenorrhea and galactorrhea.

A. Petrini
Reproductive Medicine and Ob/Gyn, Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

P. H. Chung (✉)
Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: pakchu@med.cornell.edu

Physical exam showed normal vital signs and a BMI of 23 kg/m^2. Visual field testing was normal. There were no abnormalities in skin texture or hair thinning. Reflexes were 2+ throughout. Examination of the thyroid revealed no enlargement or mass. Bilateral breast examination with compression of the nipples revealed milky white secretions. Otherwise, there was no mass or lump detected on palpation. Pelvic exam revealed normal external female genitalia, well-estrogenized vaginal mucosa, and a normal appearing cervix. Uterus was of normal size, and there were no adnexal masses on bimanual exam. Transvaginal ultrasound showed normal uterus with a 9.5 mm endometrial thickness. Bilateral ovaries were of normal size and morphology.

Urine pregnancy test was confirmed as negative. Laboratory evaluation returned with a TSH of 2.3 mIU/L and normal free T4. Prolactin level, which was sent after physical examination, was found to be 48 ng/mL. A repeat morning fasting serum prolactin was 56 ng/mL. An MRI of the brain with contrast was performed, which showed a 7 mm pituitary microadenoma.

She was treated with initiation of oral cabergoline 0.25 mg twice weekly. Within 2 weeks, she became euprolactinemic and noted a reduction in galactorrhea. Spontaneous menses returned 3 weeks later. After menses, ovulation was documented. The couple began their fertility evaluations, which revealed normal anti-Mullerian hormone level, hysterosalpingogram (HSG), and semen analysis. Despite her history of ruptured appendix, fallopian tubes spilled dye readily on HSG. Given the above assessment and resumption of ovulatory cycles with continuation of cabergoline, the patient started natural cycle monitoring with timed intercourse. In 4 months, she became pregnant.

Discussion

Prolactin, a 23 kDa polypeptide, is an anterior pituitary hormone primarily responsible for the initiation and maintenance of lactation. It also plays a central role in a variety of reproductive functions, mammary development, and immune response [1]. Prolactin secretion is predominantly controlled by the tonic inhibition of hypothalamic dopamine, which traverses the portal venous system to act upon pituitary lactotroph D2 receptors. A second signal is stimulatory provided by thyrotropin-releasing hormone (TRH), histamine, vasoactive intestinal peptide, epidermal growth factor, and hypothalamic peptides. Hyperprolactinemia may present with classic findings of galactorrhea [2] in the setting of amenorrhea as in our patient. However, patients may more frequently present with oligomenorrhea, infertility, decreased bone mass, or can simply be asymptomatic [3].

Causes of hyperprolactinemia can be physiological, pathological, or idiopathic in origin. Physiologic hypersecretion can be due to pregnancy, lactation, stress, chest wall stimulation, breast manipulation, meals, and lack of sleep. Pathologic causes include pituitary hyperproduction (prolactinoma, acromegaly, Cushing's disease, Addison's disease, etc.), hypothalamic–pituitary stalk compression

(craniopharyngioma, empty sella, Rathke's cyst, etc.), and systemic diseases (hypothyroidism, chronic renal failure, ectopic production, cirrhosis, seizure disorders, herpes zoster, tuberculosis, etc.). Hypothyroidism can cause hyperprolactinemia, as low free T4 feedback signals the hypothalamus to increase TRH. In addition to enhancing production of TSH, TRH stimulates prolactin release. About 3–10% of women with PCOS have coexistent hyperprolactinemia [4]. Drug-induced hyperprolactinemia involves those that act as dopamine antagonists such as methyldopa, clonidine, metoclopramide, tricyclic antidepressants, and antipsychotics such as risperidone. Lastly, up to 40% of cases are idiopathic.

Prolactinomas account for 30% of functioning pituitary tumors and are one of the most frequent causes of chronic hyperprolactinemia (50%). They are described as either microadenomas (less than 10 mm in size) or macroadenomas (10 mm or larger). About 40% of individuals with hypothyroidism and 30% with renal failure have mild hyperprolactinemia. Acromegaly can be manifested by co-secretion of prolactin with growth hormone.

Diagnostic considerations should first rule out secondary causes of hyperprolactinemia as listed above. The patient history must initially be assessed. This patient lacked external or historical factors that might cause hyperprolactinemia. Serum prolactin levels vary between 5 and 25 ng/mL in females, although physiological and diurnal variations occur [5]. Initial nonfasting serum prolactin on this patient was elevated, thus a repeat with fasting in the morning was performed and remained high. Her medical history was overall uncomplicated, and while she was started on a selective serotonin reuptake inhibitor, escitalopram was not expected to cause increased prolactin levels. As galactorrhea can be normal in pregnancy, a urine or serum pregnancy test ruled out this cause for her secondary amenorrhea. A normal serum TSH and free T4 excluded hypothyroidism. A normal creatinine level within the basic metabolic panel assures normal renal function. If these tests do not reveal an obvious etiology, regardless of the degree of hyperprolactinemia, imaging of the hypothalamic–pituitary area is indicated. An MRI of the brain with gadolinium enhancement is the appropriate next step. An MRI may be normal but usually reveals a micro- or macroadenoma of the pituitary. Both forms of pituitary adenomas commonly arise from the lateral wings of the anterior pituitary where lactotrophs are located. However, other possible MRI findings include empty sella syndrome, other forms of hypothalamic/pituitary tumor, or any pathology that compresses onto the pituitary stalk. In our Center, craniopharyngiomas have been diagnosed in patients with similar presentation as in this patient. Since renal adenomas associated with Cushing's disease can secrete prolactin, an additional work-up for Cushing's disease must be considered in those with clinical features compatible with the disease. Compression from a prolactinoma may also diminish the secretion of other anterior pituitary hormones. Therefore, measuring serum gonadotropins, ACTH, growth hormone, or antidiuretic hormone is clinically relevant. In patients with a longer duration of amenorrhea due to hyperprolactinemia, it is prudent to consider DEXA screening for possible decrease in bone mineral density secondary to diminished estrogen.

Generally, mild elevation in prolactin (25–50 ng/mL) will not cause symptoms, whereas levels from 50 to 100 ng/mL will begin to manifest in menstrual cycle irregularities. With a prolactin of 100 ng/mL or greater, overt hypogonadism and thus hypoestrogenism may be present from interference in GnRH pulsatility. A prolactin level above 200 ng/mL should increase the suspicion for a pituitary macroadenoma.

The management of hyperprolactinemia depends upon the underlying etiology. Systemic disorders, drugs, or stalk processes will require their underlying cause to be addressed and treated, after which prolactin will typically be normalized and symptoms of hyperprolactinemia, including galactorrhea, will be corrected accordingly.

Treatment of hyperprolactinemia due to an idiopathic reason, micro or macroadenoma, should start with medications and focus on the ultimate goals of treatment. For those not considering pregnancy, treatment should aim at normalizing serum prolactin and facilitating return of normal menses while also incorporating contraception. For those attempting to conceive, as in this patient, after prolactin levels return to normal, ovulation pattern should be evaluated. Fertility work-up including assessment of ovarian reserve, hysterosalpingogram, and semen analysis will be required. If ovulation does not occur readily, ovulation induction can be considered.

Microadenomas may regress spontaneously and do not often progress to macroadenomas (<7%). Even macroadenomas or patients with visual field deficits are not contraindications to start with medical therapy. The mainstay for normalizing prolactin level, as in our patient, is to utilize a dopamine agonist such as bromocriptine or cabergoline. Bromocriptine, a lysergic acid derivative, is a strong dopamine agonist that will directly inhibit prolactin secretion. The starting dose of bromocriptine is usually 2.5–5 mg/day. In 80% of women, normal menses and ovulation will be restored. It is important to note that side effects of these agents, including orthostatic hypotension, nausea, and headache, are not uncommon but can be reduced by starting with a lower dosage and/or administering the medications at night or even vaginally. Fewer side effects are generally seen in cabergoline, which is administered at 0.25 mg twice per week. Both agents are considered safe to use while trying to conceive.

With medications, prolactin levels should be measured in 4–6 weeks in order to titrate the appropriate maintenance dosage and achieve euprolactinemia. If fertility is not desired, microadenomas or idiopathic hyperprolactinemia can be monitored with yearly serum prolactin measurement after euprolactinemia is achieved. Oral contraceptives can be used as birth control as well as to avoid potential unopposed estrogen with anovulation. MRI needs to be repeated only if symptoms worsen or prolactin levels increase. Macroadenomas, on the other hand, should be first treated with initiation of dopamine agonists, which will decrease not only prolactin level but also tumor size [6]. Visual field defects can likely be reversed. Prolactin levels and repeat MRI are used to monitor the efficacy of treatment. If prolactin level and/or symptoms such as headache or visual disturbance are not responsive or refractory to medications, surgical intervention may be required [7]. Transnasal/transsphenoidal microexcision of the prolactinoma is the surgical treatment of choice. Radiation

therapy is only reserved for nonsurgical candidates, patients who fail to respond to surgery, or cases where complete resection is not possible [8]. Patients have to be counseled about the risks of hypopituitarism either with surgery or radiation treatment.

In conclusion, this young patient presents with classic symptoms of hyperprolactinemia due to a pituitary microadenoma. Generally, the diagnosis for elevated prolactin levels is straightforward and treatment is largely successful. However, it is important as reproductive physicians that the full clinical and pathological relevance of hyperprolactinemia is considered before treatment begins.

References

1. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. Physiol Rev. 2000;80(4):1523–631.
2. Tolis G, Somma M, Van Campenhout J, Freisen H. Prolactin secretion in sixty-five patients with galactorrhea. Am J Obstet Gynecol. 1974;118(1):91–101.
3. Taylor HS, Lubna P, Seli E. Amenorrhea. In: Speroff's clinical gynecologic endocrinology and infertility. 9th ed. Philadelphia, PA: Wolters Kluwer; 2020. p. 343–94.
4. Minakami H, Abe N, Oka N, Kimura K, Tamura T, Tamada T. Prolactin release in polycystic ovarian syndrome. Endocrinol Jpn. 1988;35(2):303–10.
5. Melmed S, Jameson JL. Disorders of the anterior pituitary and hypothalamus. In: Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL, editors. Harrison's principles of internal medicine. 16th ed. New York, NY: McGraw Hill; 2008. p. 2076–97.
6. Essais O, Bouguerra R, Hamzaoui J, Marrakchi Z, Hadjri S, Chamakhi DS, et al. Efficacy and safety of bromocriptine in the treatment of macroprolactinomas. Ann Endocrinol (Paris). 2002;63:524–31.
7. Casanueva FF, Molitch ME, Schlechte JA, Abs R, Bonert V, Bronstein MD, et al. Guidelines of the Pituitary Society for the diagnosis and management of prolactinomas. Clin Endocrinol. 2006;65(2):265–73.
8. Tsagaraki S, Grossman A, Plowman PN, Jones AE, Touzel R, Rees LH, et al. Magavoltage pituitary irradiation in the management of prolactinomas: long-term follow-up. Clin Endocrinol. 1991;34(5):399–406.

Chapter 12
Thyroid and Reproduction

Melissa D. Katz

Case

Alicia is a 39-year-old woman with primary infertility and borderline thyroid function tests (TFTs). She has tried to conceive unsuccessfully for the past 18 months and is considering undergoing assisted reproduction. There is no significant past medical history with the exception of psoriasis, which has been treated exclusively with topical preparations. Otherwise, she is taking prenatal vitamins daily. Menarche occurred at age 13, and although she took oral contraceptives pills (OCP) from her early 20s until a year and a half ago, she recalls having regular monthly menstrual cycles when she was not on OCP. Her family history is significant for hypertension in both parents and systemic lupus erythematosis in her sister. She feels well and denies fatigue or any recent change in weight, bowel function, and temperature tolerance.

On examination, she is a well appearing woman and except for a few psoriatic patches on her elbows and palms, her examination is unremarkable otherwise. Her thyroid gland is not enlarged, tender, or nodular. Two weeks ago, her thyrotropin (TSH) was 4.09 mIU/L (0.55–4.78 mIU/L) with a free T4 of 1.2 ng/dL (0.9–1.8 ng/dL). A more recent thyroid test profile revealed a TSH of 4.62 mIU/L with a free T4 of 1.1 ng/dL.

Subclinical hypothyroidism was diagnosed. Her psoriasis and infertility certainly qualify her as a candidate for screening for hypothyroidism. Further testing demonstrated positive thyroid peroxidase (TPO) antibodies. Given her desire to conceive, every effort to normalize her thyroid function is important. Therefore Alicia was started on 50 µg levothyroxine per day aiming for a TSH <2.5 mIU/L which is the first trimester-specific normal range.

M. D. Katz (✉)

Department of Medicine, Division of Endocrinology, Weill Medical College of Cornell University, New York, NY, USA

e-mail: mdkatz@med.cornell.edu

Discussion

Interpretation of Thyroid Function Tests (TFTs)

Women like Alicia are commonly encountered by medical and reproductive endocrinologists, and obstetricians and gynecologists. Their TFTs are within the acceptable normal range for the general nonpregnant population. However, since the fetal thyroid does not manufacture thyroid hormones, thyroxine (T4), and triiodothyronine (T3), until 18–20 weeks gestation, women contemplating pregnancy who have possible thyroid dysfunction should be viewed in a different context. The maternal contribution to bioavailable thyroid hormone is paramount to ensure that fetal neurodevelopment proceeds properly. Although the fetal thyroid gland makes increasingly larger amounts of thyroid hormone from the second trimester on, studies show that the mother's contribution is essential until delivery.

Due to physiologic changes that accompany pregnancy, it is imperative to use population and trimester-specific normal ranges when evaluating TFTs in a pregnant woman. The first trimester reference range is appropriate for women planning to conceive and those diagnosed with infertility. Many laboratories publish their own trimester-specific normal values. In the absence of these, the 2017 American Thyroid Association (ATA) guidelines for pregnancy suggested a normal range of TSH at 0.1–0.4 mIU/L for weeks 7–12 [1]. In general, measuring TSH, free T4, and/or total T4 usually suffices. Total T4 values can increase by up to 50% in pregnancy. This is due to elevated estrogen levels which induce increased hepatic production of thyroid-binding globulin (TBG). Thus, more thyroid hormone is bound to TBG and inactive, necessitating increased production by the thyroid gland in order to meet the demands of pregnancy. Furthermore, human chorionic gonadotropin (hCG) stimulates thyrocytes, as there is significant homology between the beta subunits of hCG and TSH. This is responsible for the transient decrease in TSH which may be observed in early pregnancy corresponding to the highest hCG levels (Fig. 12.1). In multiple gestations, where the hCG is most elevated, the TSH can be suppressed [2].

The majority of pregnant women with previously diagnosed hypothyroidism therefore require increased doses of thyroid hormone as early as 4 weeks gestation. Ideally, hypothyroid women should have a TSH <2.5 mIU/L prior to conception. Although T3 has a role in the treatment of hypothyroidism, it is not appropriate for pregnancy, and all pregnant women should receive adequate doses of levothyroxine (LT4). Women who conceive via assisted reproductive technology (ART) often require earlier and greater augmentation of their dose due to the increased demands ART places on the hypothalamic–pituitary–thyroid axis.
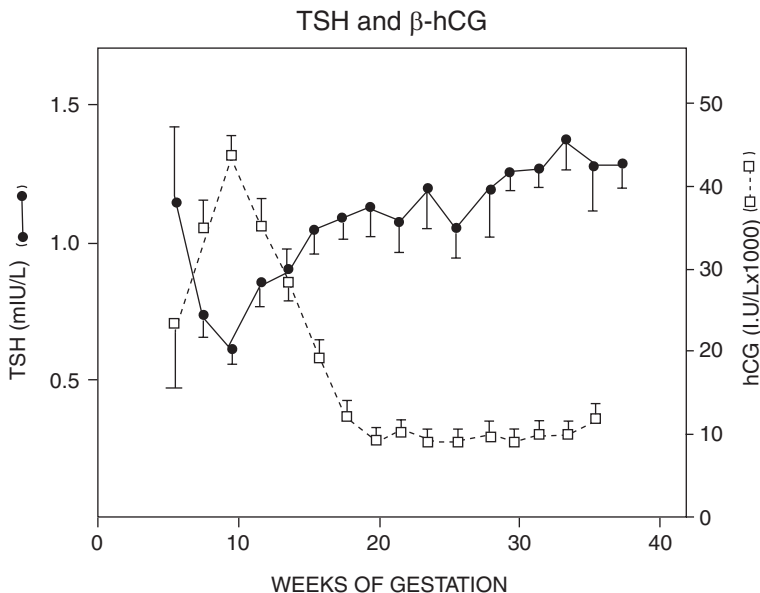
Fig. 12.1   TSH and β-hCG (Glinoer et al. JCEM 71:276, 1990)

Iodine Supplementation for Pregnancy and Lactation

Dietary iodine requirements likewise increase in pregnancy, as iodine is the substrate for thyroid hormone. The increase in glomerular filtration rate (GFR) associated with pregnancy leads to increased iodine excretion. The consequences of iodine deficiency in pregnancy include fetal hypothyroidism, goiter, and impaired neurological development. Therefore, the World Health Organization (WHO) recommends 250 μg daily iodine supplementation for pregnancy and lactation. Most American women have iodine incorporated into their diet and thus the ATA recommends a daily supplement of 150 μg iodine for pregnancy and lactation, commonly found in prenatal vitamins in the United States.

A recent meta-analysis evaluated maternal iodine status and child intelligence quotient (IQ) at 1.5–8 years in Dutch, Spanish, and British mother–child pairs. A positive association between maternal urinary iodine/creatinine ratio and mean verbal IQ was seen only up to 14 weeks gestation. This underlines the crucial role of iodine for neurological development in the first trimester.

Table 12.1 Indications for preconception or early pregnancy screening for hypothyroidism

Signs and symptoms of hypothyroidism
Residence in an iodine insufficient region
Personal or family history of hypothyroidism or thyroid autoimmunity
Infertility
Recurrent miscarriage
History of preterm birth
Type 1 diabetes mellitus or other autoimmune disorder
Goiter
History of head or neck radiation treatment
History of thyroid surgery
History of lithium or amiodarone usage

Screening for Maternal Hypothyroidism

It has been well established that overt maternal hypothyroidism can cause oligo- or amenorrhea. Overt hypothyroidism (TSH above normal range and free T4 below normal range) complicates up to 5% of pregnancies and can also be associated with adverse outcomes including pregnancy loss, preterm birth, low birth weight, and neurodevelopmental consequences such as low IQ in the offspring. However, there is a lack of consensus regarding screening asymptomatic women for hypothyroidism as intervention trials have not unanimously shown benefit. Therefore, universal screening of asymptomatic women either prior to or during pregnancy remains controversial and is not recommended by the American College of Obstetricians and Gynecologists (ACOG) or the Endocrine Society. Risk factors listed below warrant screening for hypothyroidism. Pre-conception or early pregnancy screening decisions should be made on an individual basis (Table 12.1).

Maternal Subclinical Hypothyroidism

Several randomized studies have evaluated whether screening and treatment of asymptomatic pregnant women with subclinical hypothyroidism (elevated TSH, normal free T4), as in Alicia's case would be beneficial. Subclinical hypothyroidism occurs in up to 10% of pregnancies and similar to hypothyroxinemia (normal TSH, low free T4), can be associated with miscarriage, preterm delivery and diminished cognitive ability in the offspring [3]. Among the larger and more recent studies, a predominantly Welsh cohort of pregnant women with a mean gestational age of 12 weeks 3 days was screened for subclinical maternal hypothyroidism and hypothyroxinemia [4]. Cognitive development, as measured by IQ at 3 and 9 years did not vary between the 390 children whose mothers were treated with LT4 compared

with the 404 children whose mothers did not receive treatment, at a median gestational age of 13 weeks, 3 days. Likewise, an American study of 339 pregnant women with subclinical hypothyroidism and 263 pregnant women with hypothyroxinemia, in which LT4 was initiated at a median gestational age of 16 weeks 4 days and 18 weeks, respectively, failed to show any difference in pregnancy outcome or IQ at 5 years [2]. A confounding factor in both these studies may be that the intervention occurred too late.

Thyroid Autoimmunity

In the United States and other iodine replete regions, the main etiology of hypothyroidism is thyroid autoimmunity (Hashimoto's thyroiditis), in contrast to iodine deficiency, which is the prevailing cause in developing areas. The thyroid antibodies involved in Hashimoto's thyroiditis are thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TGAb). Most reproductive studies have focused on the former. Women of reproductive age with overt hypothyroidism should be treated with thyroid hormone. Euthyroid pregnant women with TPOAb are at greater risk for adverse pregnancy outcome, specifically miscarriage and pre-term birth, than TPOAb negative women; however, treatment is controversial. TPOAb-positive women comprise up to 18% of all pregnant women and may lack adequate thyroid hormone reserve, rendering them incapable of appropriately increasing production of thyroid hormone once they conceive. Studies have demonstrated that the risk of miscarriage is doubled in TPOAb-positive women [5]. Therefore, the ATA recommends that all pregnant women with a TSH >2.5 mIU/L, be evaluated for TPOAb. Although insufficient evidence exists for the ATA to either recommend or not recommend LT4 for euthyroid TPOAb-positive pregnant women, their guidelines do note that the risk of adverse pregnancy outcomes, especially pregnancy loss and preterm birth, are increased with a TSH >2.5 mIU/L and TPOAb positivity. Monitoring for the development of hypothyroidism as often as monthly was recommended.

A recently published study from the United Kingdom investigated whether administering LT4 to euthyroid TPOAb-positive women with a history of recurrent pregnancy loss or infertility, would result in a higher live birth rate [6]. A total of 952 pregnant women were randomized to the treatment and placebo groups. LT4 was not shown to improve live birth rate; however, the ATA guidelines state that use of LT4 in euthyroid TPOAb-positive women with a prior history of pregnancy loss is reasonable. A meta-analysis, which looked at subclinical hypothyroidism, hypothyroxinemia, and TPOAb positivity, showed that all were significantly associated with an increased risk of preterm birth [7] (Fig. 12.2). The ATA recommends treatment with LT4 titrated to a TSH <2.5 for women with subclinical hypothyroidism who are undergoing intracytoplasmic sperm injection (ICSI) or in vitro fertilization (IVF).
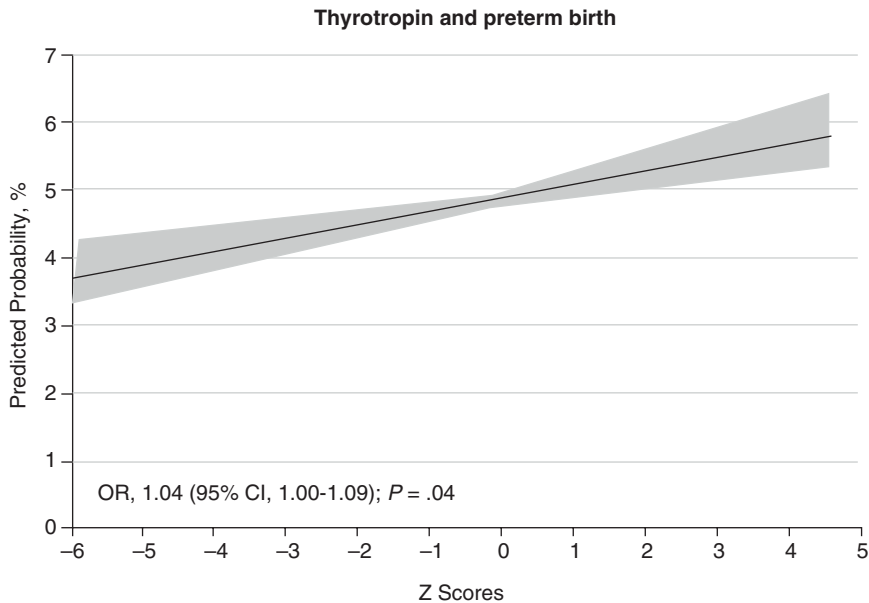
Fig. 12.2 The Consortium on Thyroid and Pregnancy—Study Group on Preterm Birth (JAMA 2019; 322(7):632–41)

Maternal Hyperthyroidism: Diagnosis

Hyperthyroidism complicates pregnancy less frequently than hypothyroidism. The TSH is decreased with a free T4 and/or free T3 above trimester specific ranges. As previously discussed, TFTs can be difficult to interpret during pregnancy, confounding the diagnosis of hyperthyroidism. A low TSH may prevail due to the elevated hCG stimulating the TSH receptor. Graves' disease is the most common cause of hyperthyroidism in women of reproductive age. It is due to antibodies which stimulate the TSH receptor (TRAb), namely thyroid stimulating immunoglobulin (TSI) and thyrotropin binding inhibitory immunoglobulin (TBII). Clinically, goiter and orbitopathy with palpitations, tremors, hyperdefecation, heat intolerance, and weight loss may result. More common than Graves' during pregnancy is transient gestational thyrotoxicosis and hyperemesis gravidarum, both of which typically improve during the second half of pregnancy. Distinguishing between Graves' disease and transient gestational thyrotoxicosis is mandatory, as the treatment differs. T3 values are often disproportionately higher than T4 levels in Graves' disease. Antithyroid drugs (ATD) are not indicated for transient gestational thyrotoxicosis, whereas these medications and surgery, are reasonable options for women with Graves' disease who are either pregnant or contemplating pregnancy. Graves' disease often abates during the latter half of pregnancy, only to flare postpartum. Measurement of TRAb, which are positive in approximately 95% of Graves' patients, may be necessary to distinguish between these two diagnoses. Pregnant

women with a history of Graves' disease should also be evaluated for TSI every trimester, as these antibodies cross the placenta and cause neonatal hyperthyroidism at a rate of approximately 20%.

Maternal Hyperthyroidism: Treatment

Untreated Graves' disease during pregnancy is associated with miscarriage, premature labor, low birth weight, stillbirth, preeclampsia, and maternal heart failure. It is always best to definitively treat hyperthyroidism prior to conception. Due to the fact that antithyroid drugs cross the placenta and the fetus is especially sensitive, the lowest possible dose should be administered to avoid fetal hypothyroidism and goiter, maintaining the mother mildly hyperthyroid. Some women can therefore be closely monitored every 4 weeks without treatment. For symptomatic control, prior to ATD taking effect, low-dose beta-blockers, specifically propranolol or metoprolol can briefly be used.

Two ATD, known as thionamides, are available in the United States, propylthiouracil (PTU) and methimazole (MMI). Due to the fact that liver toxicity is greater with PTU, MMI is preferred with the exception of the first trimester of pregnancy. This is because the teratogenic effects of these medications are less severe with PTU. These include aplasia cutis, a scalp defect and more serious abnormalities such as esophageal and choanal atresia, omphalocele, and omphalomesenteric duct anomalies. Agranulocytosis is another potentially life-threatening side effect of thionamides, and therefore the complete blood count (CBC) needs to be monitored in addition to liver function tests (LFTs). Thus, maternal considerations also mandate using the lowest possible dose. Initial thionamide doses are generally the highest and are typically halved within 2–3 weeks to 25–50 mg PTU bid or 2.5 mg methimazole daily, depending on trimester and severity of hyperthyroidism. Low doses of MMI are reportedly safe for nursing women.

In cases of intolerance to thionamides, radioiodine treatment is contraindicated for a minimum of 6 months prior to and during pregnancy. Although usually not recommended, thyroidectomy is a reasonable option for women with Graves' disease who are planning pregnancy, especially those with diminished ovarian reserve. Thyroidectomy is optimally performed during the second trimester if necessary.

Postpartum Thyroiditis

Postpartum thyroiditis usually entails an initial hyperthyroid phase beginning shortly after delivery with a duration of approximately 2–4 months. It often remains undiagnosed as symptoms suggestive of hyperthyroidism may be common postpartum. It is a painless inflammation of the thyroid gland and typically progresses to hypothyroidism, approximately 3–12 months after delivery. Permanent

hypothyroidism is most common in women with TPOAb, although some women revert to euthyroidism. The incidence may be as high as 20%, and it may recur in subsequent pregnancies. The hyperthyroid phase may need to be distinguished from Graves' disease which can present postpartum. Levels of T3 and TRAb can be useful. Symptomatic treatment with beta-blockers usually suffices for the hyperthyroid phase, whereas the hypothyroid phase should be treated with thyroid hormone. As it can take over a year to determine whether a woman with postpartum thyroiditis requires lifelong thyroid hormone treatment, it is often wise to continue LT4 in women who are planning a subsequent pregnancy relatively soon. Postpartum thyroiditis can also occur after miscarriages, abortions, and ectopic pregnancies.

Summary

1. Trimester and population-specific normal ranges for TFTs musts be used during pregnancy.
2. The majority of pregnant women with hypothyroidism require increased doses of LT4 throughout pregnancy.
3. Pregnant women should take a prenatal vitamin with 150 μg iodine.
4. The decision whether to screen for hypothyroidism prior to and during pregnancy should be individualized.
5. Adverse pregnancy outcomes including loss and preterm birth are associated with a TSH >2.5 mIU/L and TPOAb positivity.
6. LT4 should be titrated to a TSH <2.5 mIU/L for women with subclinical hypothyroidism undergoing ICSI and IVF.
7. Maternal Graves' disease should be treated with the lowest effective dose of PTU during the first trimester, and the lowest effective dose of MMI thereafter, if needed.
8. Transient gestational thyrotoxicosis, which is more common than Graves' should not be treated with ATD.

References

1. Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, et al. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease during pregnancy and the postpartum. Thyroid. 2017;27(3):315–89.
2. Glinoer D, de Nayer P, Bourdoux P, Lemone M, Robyn C, van Steirteghem A, et al. Regulation of maternal thyroid during pregnancy. J Clin Endocrinol Metab. 1990;71(2):276–87.
3. Casey BM, Thom ER, Peaceman AM, Varner MW, Sorokin Y, Hirtz DG, et al. The Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Treatment of subclinical hypothyroidism or hypothyroxinemia in pregnancy. N Engl J Med. 2017;376(9):815–25.

4. Lazarus JH, Bestwick JP, Channon S, Paradice R, Maina A, Rees R, et al. Antenatal thyroid screening and childhood cognitive function. N Engl J Med. 2012;366(16):493–501.
5. Negro R, Schwartz A, Stagnaro-Green A. Impact of levothyroxine in miscarriage and preterm delivery rates in first trimester thyroid antibody positive women with TSH less than 2.5mU/L. J Clin Endocrinol Metab. 2016;101(10):3685–90.
6. Dhillon-Smith RK, Middleton LJ, Sunner KK, Varner MW, Sorokin Y, Hirtz DG, et al. Levothyroxine in women with thyroid peroxidase antibodies before conception. N Engl J Med. 2019;380(14):1316–25.
7. The Consortium on Thyroid and Pregnancy-Study Group on Preterm Birth. Association of Thyroid Function Test Abnormalities and Thyroid Autoimmunity with Preterm Birth: a systematic review and meta-analysis. JAMA. 2019;322(7):632–41.

Chapter 13
Infertility Evaluation

Owen Davis

Case

A couple presented with an 8-month history of primary infertility and no prior fertility evaluation. The female partner was 38 years old and nulligravid, and her male partner was also 38 and denied fathering any prior pregnancies. They had been engaging in unprotected intercourse of an average of two to three times per week and were trying to have intercourse every other day at mid-cycle. Previously they had used condoms for contraception.

The female partner indicated that her menarche had occurred at age 14 and that her cycles were regular, occurring every 24–25 days. She denied a history of sexually transmitted infections (STIs). She did have a history of one abnormal PAP test with positive HPV when she was in her 20s but with a normal colposcopy at that time and normal PAP tests ever since. She regularly experienced some premenstrual bloating and mild dysmenorrhea, which did not require analgesics. Her medical history was unremarkable except for mild irritable bowel syndrome managed through diet and lifestyle modifications. Her surgical history was positive for prior wisdom tooth extraction. Her only current medication/supplements consisted of prenatal vitamins, and she denied any allergies. She denied smoking cigarettes or using recreational drugs and consumed an average of 1–2 alcoholic beverages per week. Her family history was noncontributory.

Her partner had a negative medical history; his surgical history was positive for treatment of a wrist fracture. His current medications/supplements included a daily multivitamin and occasional tadalafil for situational erectile dysfunction with satisfactory results and no ejaculatory dysfunction. He denied any allergies. He

O. Davis (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: okdavis@med.cornell.edu

indicated that he smoked cigars on average once per month, denied using recreational drugs, and consumed 3–4 alcoholic beverages per week. His family history was non-contributory.

Physical examination of the female partner revealed that she was 64 in. tall and weighed 140 lb (BMI 24 kg/m^2) and her vital signs were normal. Breast and pelvic examination were both within normal limits, and she had no evidence of thyromegaly, hirsutism, or galactorrhea. As her last menstrual period had been 18 days prior, both progesterone and anti-Mullerian hormone (AMH) levels were sent. Her progesterone level returned at 8 ng/mL, and her AMH was 1.8 ng/mL. She was scheduled to have a hysterosalpingogram (HSG) performed after her ensuing menses, and her partner was given instructions for scheduling a semen analysis.

The semen analysis report indicated an ejaculated volume of 2.5 mL, concentration of 22 million/mL, total motility of 50% (progressive motility of 40%), and sperm morphology of 5% (Kruger criteria). His partner contacted the office after her ensuing menstrual period, and an HSG was performed on the sixth day of her cycle. Upon instilling a water-soluble contrast medium, her uterine cavity was seen to have a normal contour without evidence of filling defects. Opacification of her fallopian tubes revealed mild–moderate distal tubal dilation consistent with bilateral hydrosalpinx and no intraperitoneal spillage of the contrast. The findings were reviewed with the patient, and she was given a prescription for an appropriate prophylactic regimen of oral antibiotics to start that same day.

The findings of the evaluation were reviewed with the couple, and in vitro fertilization (IVF) was advised as their most appropriate therapeutic option. They inquired whether surgical correction of her distal tubal obstruction could be undertaken, but treatment outcome data supported IVF as the option with the highest likelihood for success. Consideration of pretreatment laparoscopic salpingectomies was discussed given the known negative impact of in situ hydrosalpinx on ongoing pregnancy rates following embryo transfer.

The couple agreed with this course of action, following discussion of the risks, benefits, and alternatives. She elected to undergo laparoscopic confirmation and resection of her hydrosalpinges, which was performed uneventfully. She subsequently underwent IVF with a gonadotropin/antagonist protocol and successfully conceived after a fresh day-5 blastocyst transfer, with an additional three cryopreserved supernumerary blastocysts.

Discussion

Infertility is defined as the inability to achieve a successful pregnancy following 12 months or more of unprotected intercourse, although evaluation is warranted after 6 months in women older than 35 years, given the significant age-related decline in fertility seen in this age group. Evaluation and treatment may be initiated earlier in the event of known or suspected underlying conditions such as a history of advanced-stage endometriosis, suspected tubal disease (e.g., history of ectopic

pregnancy, pelvic inflammatory disease [PID]), irregular cycles, amenorrhea, or a known male factor.

A thorough medical history should be elicited. The duration of infertility should be ascertained; primary infertility is diagnosed in the absence of prior pregnancies. In cases of secondary infertility, the outcomes of previous pregnancies should be noted. Where applicable, any prior fertility evaluation and treatments should be reviewed. A detailed menstrual history encompasses cycle length and regularity and the presence or absence of molimina and dysmenorrhea. The timing and frequency of intercourse should be discussed. A general medical and surgical history should be obtained to establish whether there are any other coexisting chronic or acute conditions and should include specific inquiry regarding a possible history of STIs and/or PID, prior abdominal or pelvic surgeries, or evidence of endocrinopathies (hirsutism, galactorrhea, history of thyroid disease). Any current medications and supplements should be documented, including whether she is taking prenatal vitamins or supplemental folic acid. The family and social histories focus on genetic diseases, congenital malformations, and substance use/abuse (tobacco, alcohol, recreational drugs). A history should similarly be obtained from the male partner, including whether he had fathered prior pregnancies and additionally with a focus on possible sexual dysfunction and relevant medical conditions and surgeries (including urologic procedures, inguinal hernia repair) in addition to his family and social history.

The physical examination of the female partner includes vital signs and determination of her body mass index (BMI). The presence of hirsutism, acne, thyroid enlargement, and/or galactorrhea should be noted. A pelvic examination should be performed to assess for tenderness, uterine enlargement, or adnexal masses. Transvaginal sonography may be additionally performed to assess for possible ovarian cysts, antral follicle count, and uterine abnormalities (myomas, suspected adenomyosis).

The subsequent diagnostic evaluation should generally include assessment of ovarian function (ovulatory status and reserve), female anatomic factors (uterine and tubal), and the male factor. If indicated by the history or physical examination, further evaluation for peritoneal factors may be considered.

Normal fertility requires intact ovulatory function. Ovulatory dysfunction is diagnosed in approximately 15% of couples experiencing infertility and should be sought as part of the core fertility evaluation. If a patient has a history of regular menstrual cycles, ranging between 21 and 35 days, and experiences molimina (e.g., perimenstrual breast tenderness, bloating) and some degree of menstrual cramping, it is highly likely that she is ovulating, and it is a matter of some debate whether further documentation is necessary [1]. In addition, some patients will report detection of an LH-surge when utilizing home-based ovulation predictor tests. Nonetheless, it is reasonable to document presumptive ovulation with a peripheral serum progesterone determination performed in the expected luteal phase. A progesterone value exceeding 3 ng/mL is considered confirmatory [2]. A progesterone concentration in a higher range is not requisite, as ovarian progesterone secretion is pulsatile with significant variability throughout the day. Daily recording of the woman's basal body temperature (to

detect a thermogenic shift) and/or performing an endometrial biopsy in the luteal phase (to histologically identify and date secretory endometrium) are largely of historic interest and no longer routinely advised. When anovulation is diagnosed, further evaluation is indicated to identify an etiology such as polycystic ovary syndrome, hypothalamic amenorrhea, or other endocrinopathies in order to appropriately guide subsequent treatment.

It is appropriate to briefly address the concept of "ovarian reserve" when discussing ovarian function. Follicular atresia is a normal physiologic component of ovarian aging, with depletion of follicles and oocytes commencing at approximately 20 weeks gestation in utero and continuing until menopause. Ovarian reserve, while predictably declining with advancing chronologic age, can vary considerably between women at any given age due to different baseline follicular endowments and/or rates of atresia. While various static and dynamic tests have been utilized over the years (e.g., basal early follicular phase FSH and estradiol levels, clomiphene challenge testing), the principal biomarkers utilized in contemporary practice are the assessment of AMH levels and basal AFCs determined with transvaginal sonography. AMH levels below approximately 1.0 ng/mL and AFC <8–10 are generally taken to connote diminished ovarian reserve [3]. For the purposes of the current discussion, it should be emphasized that the utility of ovarian reserve testing is primarily for predicting treatment response to ovarian stimulation with exogenous gonadotropins for IVF; it is used both for counseling purposes and the selection of an optimal medication protocol. Measures of ovarian reserve are otherwise poorly predictive of a woman's ability to conceive; even in the context of the assisted reproductive technologies, ovarian reserve testing cannot reliably predict pregnancy or non-pregnancy following treatment.

Spontaneous conception requires at least unilateral tubal patency, and fertility may also be impaired in the setting of either congenital or acquired uterine abnormalities. Hence, an assessment of the anatomy of the upper female reproductive tract is another core component of the infertility evaluation. Several imaging modalities are available for the individual or combined assessment of uterine and tubal anatomy, ranging from radiologic procedures to 2D and/or 3D sonography, MRI, and endoscopy. In most practices, the principal techniques employed in the basic fertility evaluation are hysterosalpingography (HSG) and/or saline infusion sonography (SIS, also termed sonohysterography). The HSG, utilizing either water-based or lipid-soluble contrast, has the advantage of assessing both uterine and tubal anatomy. An HSG can identify congenital uterine anomalies (unicornuate, septate, bicornuate) in addition to the presence of uterine filling defects (polyps, myomas, intrauterine adhesions). Specifically distinguishing between a uterine septum and a bicornuate uterus may require additional testing such as sonography or a pelvic MRI to better assess the external uterine contour. The HSG is also the mainstay for assessing tubal patency

and anatomy. Unilateral or bilateral proximal or distal tubal occlusion can be identified and specifically the presence of hydrosalpinx in cases of distal obstruction. False positives are not uncommon, particularly in cases of suspected proximal tubal occlusion, as may occur with transient tubal spasm. Performance of an HSG may, in and of itself, have some therapeutic benefit in the setting of tubal patency, with enhanced pregnancy rates seen in the months following the procedure. The SIS entails the intracavitary instillation of sterile saline with concurrent ultrasound imaging. The SIS is a very sensitive modality for the detection of endometrial polyps, submucous myomas, and other uterine pathology; in contrast with the HSG, the echotexture of any identified intrauterine pathology can more specifically suggest a polyp versus submucous myoma. Tubal patency can be inferred by the accumulation of saline in the cul-de-sac during an SIS, but one cannot as clearly distinguish unilateral versus bilateral tubal patency, and the tubal anatomy is less well defined than with an HSG. If anatomic abnormalities are identified, various therapeutic options may be suggested, ranging from operative hysteroscopy for intrauterine pathology to laparoscopy for suspected endometriosis and/or pelvic adhesions and IVF for occlusive tubal disease. While routinely performed in the past, laparoscopy is no longer recommended as a standard diagnostic component of the female infertility evaluation, with potential exceptions being cases with identified pelvic pathology or significant symptomatology.

The fundamental initial laboratory evaluation of the male is the standardized semen analysis. The male partner is asked to produce an ejaculated semen sample after a specified abstinence interval, generally of 2–3 days. A complete analysis quantifies the volume of the ejaculate and determines the sperm concentration, percentage motility, and proportion of sperm exhibiting normal morphology. Per current World Health Organization criteria, normal semen parameters include: an ejaculate volume of at least 1.5 mL, sperm concentration of at least 15 million/mL, a sperm motility of 40% or greater, progressive motility of 32% or greater, and a morphology of 4% or greater normal forms [4]. Abnormal initial semen analysis bears repeating; when a male factor is identified, the male partner should be referred to a urologist or andrologist with a clinical focus on male infertility. Treatment options for the male can range from lifestyle modification to hormonal treatment, surgery for large varicoceles or anatomic obstruction, intrauterine insemination (IUI), IVF with intracytoplasmic injection (ICSI), or surgical sperm retrieval for use with IVF in cases of obstructive or non-obstructive azoospermia (absence of sperm in the ejaculate), depending on the underlying etiology and severity of sperm impairment.

In conclusion, in the case outlined at the outset of this chapter, a complete basic infertility evaluation revealed distal tubal occlusion (with hydrosalpinges) as the sole identifiable etiology for this couple's infertility and led to an effective therapeutic strategy, specifically IVF, to successfully bypass the underlying pathology.

References

1. Munster K, Schmidt L, Helm P. Length and variation in the menstrual cycle- a cross-sectional study from a Danish county. Br J Obstet Gynaecol. 1992;99:422.
2. Wathen NC, Perry L, Lilford RJ, Chard T. Interpretation of single progesterone measurement in diagnosis of anovulation and defective luteal phase: observations on analysis of the normal range. Br Med J (Clin Res Ed). 1984;88:7–9.
3. Ferraretti AP, La Marca A, Fauser BCJM, Tarlatzis B, Nargund G, Gianaroli L, ESHRE Working Group on Poor Ovarian Response Definition. ESHRE Consensus on the definition of "poor response" to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod. 2011;26(7):616–24.
4. WHO. Laboratory manual for the examination and processing of human semen. 5th ed. Geneva: WHO; 2010.

Chapter 14
Hypothalamic Hypogonadism

David Reichman

Case

A 28-year-old G1P1 presents to her gynecologist with no menses over the last 4 months. She was previously trying to conceive for about 15 months and is distraught about the impact her lack of menses may have on her fertility. A home urine pregnancy test has been repeatedly negative. Her gynecologic history is notable for previously cyclic menses every 31–33 days. She conceived easily and had a normal vaginal delivery of her healthy daughter 4 years ago. She breastfed for 1 year and had resumption of menses when her child was 6 months of age. Her past medical history is notable only for mild gastroesophageal reflux and a 2-year history of anorexia in college, which resolved through psychological therapy and working with a nutritionist. Her only surgery was wisdom teeth removal as a teenager. She has recently suffered the death of her mother who had a long battle with small-cell lung cancer.

On exam, the patient is well appearing and in no distress. She is 5′8″ tall and weighs 120 lb (BMI 18.24 kg/m^2). Pelvic exam is unremarkable, and transvaginal ultrasound reveals normal uterine anatomy, a 4 mm homogenous endometrial stripe, and no adnexal masses. Several follicles were noted in each ovary in various phases of early follicular development. Serologic examination reveals an FSH of 1.6 mIU/mL, LH of 2.1 mIU/mL, E_2 of 25 pg/mL, and P_4 of 0.2 ng/mL. Serum bhCG level is negative. Anti-Mullerian hormone level is 3.5 ng/mL. Thyroid function, prolactin level and serum androgens are within normal limits.

On further discussion with the patient, she reports that she has been exercising daily to help cope with her grief from her mother's death. She has been taking four vigorous cycling classes, three Pilates sessions, and jogging 20 miles/week. About

D. Reichman (✉)

Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: der2005@med.cornell.edu

8 months ago she initiated a new diet that involves eating only an avocado for breakfast, a vegetarian salad for lunch, and a protein with steamed vegetables for dinner. We discussed with her a diagnosis of functional hypothalamic amenorrhea and various ways to address the causes of the dysfunction. After several months of increased caloric intake and reduced exercise regimen, the patient resumed menses which were occurring every 30–40 days. To ensure regular ovulation, she was started on monthly low-dose clomiphene citrate (50 mg day 3–7) and was monitored by blood work and ultrasound each cycle. Human chorionic gonadotropin (hCG) trigger was given when follicles were mature. Timed intercourse instructions were reviewed. She conceived after 3 months of such treatment.

Discussion

Functional hypothalamic hypogonadism is one of the most common causes of secondary amenorrhea, and most frequently originates from low energy availability due to a combination of inadequate caloric intake and excessive energy expenditure. The so-called female athlete triad refers to excessive exercise/disordered eating, irregular or absent menses, and bone mineral density loss. Such individuals may have lower circulating levels of leptin, which acts as a signal from peripheral adipocytes to the CNS to reduce gonadotropin pulsatility [1].

Organic, nonfunctional causes of hypothalamic hypogonadism (rarer than functional cases) include central mass lesions, pituitary infarction, prior pituitary surgery, or prior infection or radiation. Such causes frequently present with panhypopituitarism rather than the isolated hypogonadism like our patient illustrated above. Regardless of etiology, hypothalamic hypogonadism is characterized by decreased GnRH pulsatility, abnormal or absent follicular development, and insufficient mid-cycle luteinizing hormone levels, leading to anovulation and associated low estradiol levels.

Diagnosis of hypothalamic hypogonadism is triggered by the presence of amenorrhea or oligomenorrhea with associated low serum levels of FSH, LH, and estradiol. History should focus on precipitating factors such as increased stress, physical exercise, decline in nutritional status, or a combination of such factors. Pertinent questions include history of eating disorders, recent weight loss, amount of exercise, psycho-social stressors, and dietary intake. The patient's previous BMI at which she exhibited cyclic menses should be established. For a diagnosis of functional hypothalamic amenorrhea, other organic causes should first be excluded based on history, exam, and pituitary imaging if indicated.

Biochemical testing should include human chorionic gonadotropin (hCG) to exclude pregnancy, prolactin to detect hyperprolactinemia, thyroid-stimulating hormone, and free T4 levels to exclude thyroid disease, in addition to FSH, LH, and E2. Thin women with polycystic ovary syndrome (PCOS) can present a diagnostic challenge—serum androgens should be sent in the setting of clinic evidence of hyperandrogenism to help differentiate between thin-PCOS and mild hypothalamic

hypogonadism. AMH may also be useful here as it is frequently elevated in individuals with PCOS, but a high AMH is by no means diagnostic. If medical disorders triggering a hypothalamic state are suspected, complete blood counts, inflammatory markers (ESR, CRP), and basic metabolic panel should also be done.

Dynamic testing is not required to make the diagnosis but can be a low-cost modality for determining whether PCOS or functional hypothalamic hypogonadism is a more likely diagnosis. Failure to exhibit a withdrawal bleed to a progestin challenge or a thin endometrial stripe on pelvic ultrasound suggests estrogen deficiency as seen in hypogonadism. Measurement of serum estradiol in conjunction with serum gonadotropins, however, often provides enough data. MRI of the pituitary is indicated in women with hyperprolactinemia, unexplained hypogonadotropic hypogonadism, or localizing CNS symptoms, such as new onset headaches or visual changes. Bone mineral density assessment should be conducted in patients with >6 months amenorrhea or those in whom severe malnutrition is suspected.

Treatment in patients with functional hypothalamic hypogonadism should focus on anovulatory infertility (if patients desire conception), and consequences of hypoestrogenemia such as sexual dysfunction and low bone mineral density [2]. Once a diagnosis is confirmed, primary management should involve counseling the patient regarding the underlying condition's pathophysiology and how it can be addressed through lifestyle modifications. Often this means educating patients regarding target body weight goals and adequate caloric intake for the amount of energy being expended on a daily basis. Reduction in exercise intensity, duration, and frequency, along with dietary changes to increase caloric intake, is often sufficient to bring about resumption of menses [3]. Individuals who have a BMI <18.5 kg/m^2 and exhibit secondary amenorrhea should be instructed to increase caloric intake and decrease exercise so as to re-attain normal BMI and the weight at which they previously exhibited cyclic menses [4]. It is important to convey to significantly underweight patients that it can take several months even after achieving a normal BMI for menses to resume [5]. Return to menses sometimes requires patients to attain a weight greater than what they previously had when cyclic menses occurred.

In cases where functional hypothalamic hypogonadism is due, at least in part, to disordered eating, a multidisciplinary approach involving a psychiatrist or psychologist, a dietitian with expertise in eating disorders, and the patient's primary physician should be employed. Cognitive behavioral therapy or family-based therapy have both shown efficacy in addressing psychological issues underpinning disordered eating such as distorted body image and prior trauma [6]. Where necessary, adjuvant treatment with pharmacologic therapy such as SSRIs can help in high-stress individuals where therapy alone is insufficient.

Fertility is impaired in individuals with hypothalamic hypogonadism due to anovulation. Where possible, treatment should begin with the same lifestyle modification to increase BMI, reduce physical activity, and improve nutritional intake to allow for restoration of normal GnRH pulsatility. Overly thin patients should be apprised of the risks of conceiving and carrying a pregnancy with an extremely low BMI, which has been associated with higher miscarriage rates and low birth weight [7]. When lifestyle modification is inadequate or falls short of resuming normal

cyclic menstruation, as in our patient, ovulation induction agents can be used. Whereas oral agents such as letrozole or clomiphene citrate are the mainstays of ovulation induction in amenorrheic individuals with PCOS, such agents are frequently insufficient to either induce ovulation or sustain follicular development in patients with functional hypogonadism. A trial of clomiphene citrate is reasonable in patients who do not exhibit profound gonadotropin suppression. A reasonable starting dose is 50 mg for 5 days, with careful monitoring thereafter with transvaginal ultrasonography to assess follicular development and measure serum estradiol. Frequently, such patients will mount an initial brief response that is enough to initiate follicular development but insufficient to sustain growth or potentiate an LH surge. A human chorionic gonadotropin injection can be used to evoke follicle rupture and increase luteal production of estradiol and progesterone. However some patients will be highly sensitive to these ovulation induction agents and pose a risk of developing multiple follicles. Therefore ovarian hyperstimulation syndrome and multiple pregnancies have to be avoided by careful monitoring with ultrasound examination and blood work.

In individuals who do not mount a sufficient response to oral ovulation induction agents, or who are too profoundly suppressed to attempt a trial of oral therapy, injectable gonadotropins can be employed for follicular recruitment. Use of gonadotropins is usually combined with intrauterine insemination (IUI) or IVF. The latter has the benefit of reducing the risk of multiple gestation by allowing for single embryo transfer, with the downside, however, of being more expensive and invasive to the patient. Injectable ovulation induction (IUI) requires patience on the part of both physician and patient, with careful serologic and ultrasound monitoring so that much less robust follicular recruitment can be achieved (no more than 2–3 dominant follicles). Injectable gonadotropins carry a high risk of twins or higher-order multiple gestations, and the patient must be prepared for either cycle cancellation or conversion to IVF if too high a response is encountered. To prevent such a scenario, a "low and slow" approach to dosing should be pursued. Dosing should commence at between 50 and 75 units of FSH or combined FSH/LH per day (dependent on patient BMI and antral follicle count) and not increased until at least 5–7 days of treatment has not shown an increase in estradiol or follicular growth. As an alternative to injectable gonadotropins, a GnRH pump can be employed, but unfortunately these pumps are not commercially available in the United States.

For those patients not in pursuit of fertility, and in whom lifestyle modification is not curative, therapy should focus on maintenance of bone density. Patients should be encouraged to supplement with both calcium and vitamin D. This supplementation should occur in conjunction with hormone replacement therapy using transdermal estrogen and cyclic progestin. Such therapy may also enhance overall well-being indices [8]. One patch of estradiol (0.1 mg) can be changed every 96 h, with oral medroxyprogesterone 10 mg or norethindrone 5 mg taken for 10–14 days each month to induce withdrawal and protect against the hyperplasia risk of unopposed estrogen [9]. Combined oral contraceptive pills are not recommended for hormone replacement, as they are inferior in improving bone density as compared to physiologic replacement. It is important to note that bisphosphonates are contra-indicated

in women of child-bearing age interested in subsequent fertility due to potential long-term teratogenicity.

In summary, hypothalamic hypogonadism is one of the most common causes of secondary amenorrhea and most frequently results from an energy imbalance involving either caloric restriction or over energy expenditure. After the diagnosis has been established and other organic causes ruled out, first-line therapy involves lifestyle modification to address the underlying pathophysiology. If endogenous GnRH pulsatility cannot be restored, fertility issues can be achieved with either oral ovulation induction agents or injectable gonadotropins. Last but not least, potential bone loss can be addressed via hormone replacement therapy.

References

1. Chan JL, Mantzoros CS. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. Lancet. 2005;366(9479):74–85.
2. Gordon CM, Ackerman KE, Berga SL, Kaplan JR, Mastorakos G, Misra M, et al. Functional hypothalamic amenorrhea: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2017;102(5):1413–39.
3. Warren MP, Perlroth NE. The effects of intense exercise on the female reproductive system. J Endocrinol. 2001;170(1):3–11.
4. Perkins RB, Hall JE, Martin KA. Aetiology, previous menstrual function and patterns of neuro-endocrine disturbance as prognostic indicators in hypothalamic amenorrhoea. Hum Reprod. 2001;16(10):2198–205.
5. Golden NH, Jacobson MS, Schebendach J, Solanto MV, Hertz SM, Shenken IR, et al. Resumption of menses in anorexia nervosa. Arch Pediatr Adolesc Med. 1997;151(1):16–21.
6. Michopoulos V, Mancini F, Loucks TL, Berga SL. Neuroendocrine recovery initiated by cognitive behavioral therapy in women with functional hypothalamic amenorrhea: a randomized, controlled trial. Fertil Steril. 2013;99(7):2084–91.
7. Blais MA, Becker AG, Burwell RA, Flores AT, Nussbaum KM, Greenwood DN, et al. Pregnancy: outcome and impact on symptomatology in a cohort of eating-disordered women. Int J Eat Disord. 2000;27(2):140–9.
8. Misra M, Katzman DK, Estella NM, Eddy KT, Weigel T, Goldstein MA, et al. Impact of physiologic estrogen replacement on anxiety symptoms, body shape perception, and eating attitudes in adolescent girls with anorexia nervosa: data from a randomized controlled trial. J Clin Psychiatry. 2013;74(8):765–71.
9. Ackerman KE, Singhal V, Baskaran C, Slattery M, Campoverde Reyes KJ, Toth A, et al. Oestrogen replacement improves bone mineral density in oligo-amenorrhoeic athletes: a randomised clinical trial. Br J Sports Med. 2019;53(4):229–36.

Chapter 15
Endometriosis and Infertility

Glenn Schattman

Case

A 29-year-old G0 female presents for a second opinion regarding her infertility. She states that she and her husband have been trying to conceive for the last year and ideally would like two children. Her gynecologist gave her clomiphene citrate for three cycles, and she failed to become pregnant. She was then referred to a local reproductive endocrinologist for further evaluation and treatment. Her work-up revealed an anti-Mullerian hormone (AMH) level of 4.3 ng/mL. A hysterosalpingo-gram (HSG) showed normal uterine cavity, right distal tubal obstruction without hydrosalpinx, and a patent left fallopian tube with possible peri-tubal adhesions. Her husband had a normal semen analysis. Pelvic ultrasound revealed a 4 cm right ovarian complex cyst that was most compatible with an endometrioma. Additionally, the report revealed some limited mobility of the pelvic organs consistent with pelvic endometriosis and adhesions. Her antral follicle count was approximately 25 on the left ovary, and but there were just a few scattered follicles surrounding the complex cyst on the right. On being further questioned, she remembered that at age 19 she suffered from severe dysmenorrhea and deep dyspareunia for which she was placed on birth control pills. Some relief was observed.

She was advised to proceed directly to in vitro fertilization (IVF) by her local infertility specialist to bypass the tubal factor, which was likely related to adhesive disease from endometriosis. On her first cycle, she had 14 oocytes retrieved. She was told that there was some difficulty getting access to the left ovary, and there was no oocyte retrieved from the right ovary due to the cyst. She was given a dose of IV antibiotics as there was some dark-colored fluid aspirated during the retrieval from

G. Schattman (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: glschatt@med.cornell.edu

the left ovary. Of the 14 oocytes retrieved, all were mature and ten fertilized normally with standard insemination. Only two embryos developed to the blastocyst stage, and both were biopsied and cryopreserved for preimplantation genetic testing for aneuploidy (PGT-A). PGT-A results later revealed that only one embryo was euploid; it was transferred but did not result in any pregnancy.

The patient presented to our office for a second opinion and to discuss her options. Given the size of the cyst and possibly a more severe form of endometriosis, she was advised to undergo laparoscopy to remove the cyst and evaluate/treat any pelvic disease before IVF. At surgery, she was noted to have stage 4 endometriosis with pelvic adhesions and diffuse endometriotic lesions. After lysing adhesions and removing visible disease as well as resecting the right ovarian cyst, bilateral tubal patency was also restored and confirmed on chromotubation. After surgery, she was encouraged to become pregnant naturally. After timed intercourse for 4 months, she successfully conceived and subsequently delivered a healthy full-term infant.

Discussion

Endometriosis is a common disease found in as many as 25–50% of women with infertility and up to 80% of patients with pelvic pain [1]. Whether endometriosis reduces fecundability remains controversial, although the patient described above clearly has both symptomatic endometriosis and infertility. Mechanisms for endometriosis-associated infertility include altered tubal function, altered peritoneal environment affecting efficient ovum pick up and tubal transport, impaired fertilization, ovulatory disorders with altered luteal hormone production, impaired implantation, and poor oocyte/embryo quality [2]. It is important to note that the actual mechanisms of these hypothetical etiologies for infertility remain unproven. This couple's infertility appears to be related to her tubal obstruction and adhesions, likely a result of the inflammatory condition secondary to the endometriosis. In infertile patients with no evidence of tubal disease and an otherwise normal evaluation, diagnostic laparoscopy to look for endometriosis is not warranted. Even if endometriosis is found, the improvement in monthly fecundability is not clinically significant. Two randomized controlled trials [3, 4] attempted to address if asymptomatic infertile patients with minimal or mild endometriosis (the stage of disease most commonly found at diagnostic laparoscopy in this population) would benefit from surgical ablation or excision. However, since only about 30% of asymptomatic infertile patients were noted to have endometriosis, and fewer yet will have stage 1–2 (minimal-mild) disease, the actual number of laparoscopies needed to be performed for each additional pregnancy is approximately 40! [2] Thus, diagnostic laparoscopy for unexplained infertility is not indicated.

More advanced disease, as described in our patient who had a 4 cm endometrioma, pelvic pain, and less mobile pelvic organs, is an entirely different clinical condition which should require surgery, not only for symptom relief and fertility improvement but also for pathology of the cyst to be established [5]. Laparoscopy

not only restored pelvic anatomy by removing the endometrioma, it also relieved tubal obstruction, allowing for normal conception to occur. It should be emphasized, however, surgical removal of endometriomas should be performed carefully so that normal ovarian cortical tissues will not be damaged.

Medical therapy, such as hormone suppression using a GnRH agonist, GnRH antagonist or androgen therapy will only serve to delay conception and has not been shown to improve fecundability. Therefore, it has no role in treating endometriosis-related infertility.

Since definitive or pathological diagnosis of endometriosis in asymptomatic patients requires a diagnostic laparoscopy with biopsy, one should be cognizant of the fact that some patients with unexplained infertility may in fact have endometriosis. If the infertility evaluation is otherwise normal, conventional treatments with ovulation induction and intrauterine insemination (IUI) are first indicated. The number of treatment cycles will depend on the age of the patient. Patients with diminished ovarian reserve or are older than age 35 should not procrastinate or delay treatment and should consider undergoing IVF right away or if IUI is not successful after 3 cycles.

In the patient described above, with the 4 cm adnexal mass and tubal disease, surgery rather than IVF should have been the first treatment route. Because tubal disease associated with her endometriosis is the most likely cause for her infertility, surgical intervention would restore her ability to conceive spontaneously. Her pelvic pain was also alleviated although pain relief in these patients is often only temporarily relieved by surgery. Success with IVF first without surgery would be limited in our patient due to her distorted anatomy which reduced access to her ovaries, resulting in fewer oocytes retrieved and fewer embryos available for selection for transfer. Her chances were further impacted upon by mandatory PGT-A which is not advantageous in women younger than 35 (see Chap. 32).

Even if the anatomy cannot be fully restored at the time of laparoscopy, surgery often improves symptoms and ovarian access when IVF is performed. There is no role for aspiration of endometriomas either before stimulation or at the time of oocyte retrieval, as these are very likely to recur in a short period of time. Aspiration can increase the risk of infection and will not improve ovarian response to stimulation or overall pregnancy rates [6]. Medical treatment following surgery has not been shown to improve IVF outcomes either and, as mentioned previously, will only serve to delay attempts at pregnancy. This is especially critical for older patients with an already compromised ovarian reserve.

There has been some interest in pretreating endometriosis patients who have failed to conceive in a prior IVF cycle with a GnRH agonist for 3–6 months. This treatment is based on the theory that endometriosis negatively affects oocyte quality and endometrial receptivity. Therefore it is thought that creating a hypo-estrogenic state to debulk disease may reverse this effect. Although a meta-analysis showed improved clinical pregnancy rates in the treatment arm, the extremely high rates of live births in the treatment and control groups in two of the studies make the results of this meta-analysis more difficult to interpret [7]. Further studies are needed before GnRH-a treatment prior to IVF can be routinely recommended for women with endometriosis, even in those who have had a prior failed IVF cycle.

In conclusion, endometriosis is a common disease in women with pelvic pain and/or infertility. There is very limited role for surgery to either diagnose or treat asymptomatic patients with otherwise unexplained infertility after a thorough evaluation. Patients with large ovarian endometriomas (~4 cm or larger) without pathologic diagnosis may benefit from laparoscopic surgery to remove the cyst, potentially restoring anatomy and improving access to the ovaries if IVF treatment is necessary. Our patient presented here, due to her advanced disease and symptoms, should have undergone surgery before IVF for the reasons discussed above. She may have avoided the risks and expense of the first unsuccessful IVF cycle altogether.

In patients who are already undergoing laparoscopic surgery for another indication, there is evidence to support ablating visible endometriosis discovered incidentally, as this may confer some benefit in fertility. In those patients with unexplained infertility, conventional treatments with ovulation induction and IUI, or IVF if IUI is not successful, should be the treatment of choice. Patients with documented endometriosis who undergo IVF have generally similar success rates as patients with other infertility etiologies undergoing IVF. Repeated surgeries to resect pelvic lesions or ovarian cysts should be avoided, as this will not result in improved pregnancy rates and will only serve to damage follicles and reduce ovarian reserve, thus lowering the overall probability of pregnancy.

References

1. Missmer SA, Hankinson SE, Spiegelman D, Barbieri RL, Marshall LM, Hunter DJ. Incidence of laparoscopically confirmed endometriosis by demographic, anthropometric, and lifestyle factors. Am J Epidemiol. 2004;160(8):784–96.
2. The Practice Committee of the American Society of Reproductive Medicine. Endometriosis and infertility: a committee opinion. Fertil Steril. 2012;98(3):591–8.
3. 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.
4. 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.
5. Schenken RS. Modern concepts of endometriosis. Classification and its consequences for therapy. J Reprod Med. 1998;43(3 Suppl):269–75.
6. Garcia-Velasco JA, Somigliana E. Management of endometriomas in women requiring IVF: to touch or not to touch. Hum Reprod. 2009;24(3):496–501.
7. Sallam HN, Garcia-Velasco JA, Dias S, Arici A. Long-term pituitary down-regulation before in vitro fertilization (IVF) for women with endometriosis. Cochrane Database Syst Rev. 2006;(1):CD004635.

Chapter 16
Uterine Fibroids

Rony Elias

Case

A 29-year-old nulligravid patient presents for a second opinion regarding treatment for an intramural myoma with a submucosal component. She reports a prior history of infertility and a recent biochemical pregnancy loss with her current partner. Her menstrual cycles are regular but with excessive bleeding and a duration of more than 10 days. In addition, she reports having occasional intermenstrual bleeding.

A single myoma was initially identified by her general OBGYN who performed a transvaginal sonogram. She was then referred to a reproductive endocrinologist who confirmed the location and size of the myoma by performing a saline infusion sonogram (SIS). Due to the size (5 cm) and location of the myoma (intramural), the recommendation was to perform an abdominal myomectomy and then resume natural trying for 6–12 months post-surgery.

Her past medical history is significant for chronic iron deficiency anemia. She has no prior surgical history. She has no family history of gynecologic or other cancers. She is a nonsmoker, denies illicit drug use, and consumes alcohol only occasionally. Her 30-year-old partner is healthy, never fathered any pregnancy except for the biochemical loss with the patient. His semen analysis was normal.

Her general physical exam was unremarkable with normal vital signs. She appeared well-nourished. Her pelvic exam revealed a minimally enlarged uterus to palpation. Transvaginal sonogram confirmed the presence of a 5 cm anterior wall intramural myoma with a significant submucous component. The patient was counseled about the potential impact of the myoma on her menstrual symptoms as well as fertility and miscarriages. In addition, she was explained regarding her medical

R. Elias (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: rta9002@med.cornell.edu

and surgical treatment options. In view of her desire to conceive, surgical options were discussed: abdominal, laparoscopic/robotic, and hysteroscopic myomectomy. The risks and benefits of each method were explained. More importantly, the time to recovery before she can resume attempting to conceive was discussed.

She agreed to undergo a hysteroscopic myomectomy with the proper counseling that it might require more than one session, given the size of the myoma, to completely remove it; if during surgery, resection is deemed impossible hysteroscopically, converted to an abdominal approach would have to follow. During the first hysteroscopy, more than 3 cm (around 60%) of the myoma was resected using TruClear morcellator with a 250 cc normal saline deficit. The remaining portion of the myoma could not be removed due to its depth in the myometrium. Six weeks later, she underwent another hysteroscopy. More of the myoma had protruded into the cavity; however, the procedure could not be completed due to the large fluid deficit (2400 cc normal saline) leaving approximately 10% of the myoma in situ. A month later, a third and final hysteroscopy was performed, and the remaining portion of the myoma was removed. A follow-up vaginal sonogram a few weeks later confirmed the absence of any remaining myoma with a normal endometrial stripe. The amount of her menstrual flow became normal and bleeding lasted only 4–5 days. A few months later, the patient conceived naturally and had a normal vaginal delivery of a healthy baby boy at full term.

Discussion

Uterine myomas are common benign monoclonal tumors with an estimated cumulative incidence of almost 70% and more than 80% in white and black women, respectively. More importantly, approximately 50% of premenopausal women with no prior history of myoma may have a myoma identified on vaginal sonogram [1]. In addition to ethnicity, other factors which will increase the risks of developing myomas include pre-menopausal status (tenfold), family history of myomas (threefold), and more than 5 years since last birth (two to threefold) [2]. Classically, myomas are classified into three categories by location: sub-mucosal, intra-mural, and subserosal. More recently, the International Federation of Gynecology and Obstetrics (FIGO) updated the classifications which are followed by most authorities [3].

Asymptomatic myomas in the past were typically diagnosed during routine bimanual pelvic exams. However, they needed to be of a certain size before they could be appreciated by pelvic examination. With abdominal and vaginal ultrasound readily available now, most myomas, even much smaller are diagnosed with imaging studies done at routine gynecologic visits. Due to their cost, MRIs are not usually recommended for routine use, but they are commonly ordered prior to surgery, especially in infertile patients. This is extremely helpful to delineate the uterine anatomy and more specifically the relation of myomas to the endometrium.

Depending on their location, number, and size, symptomatic uterine myomas can present with heavy bleeding, pain, and urinary and gastrointestinal symptoms.

However, it is a controversial topic about the impact of myomas on infertility and the risk of miscarriage [4]. Most authorities agree that intracavitary, submucous, or intramural myomas with submucous components have a negative impact on achieving a pregnancy and increase the risk of miscarriage [5, 6]. Large intramural and subserosal myomas can affect tubal status and be associated with tubal blockage and even hydrosalpinx after myomectomy. In addition, large myomas, regardless of their location, can prevent access to the ovaries during egg retrieval in IVF.

The impact of myomectomy on fertility, pregnancy, miscarriages, and live birth rates has been debated for many years. A recent Cochrane review on the effect of myomectomy on fertility outcomes showed that there was limited evidence to determine the role of myomectomy for infertility [7]. Another trial that looked at the impact on pregnancy rates of myomectomy performed on patients with myomas of different locations did not find any improvement compared to patients who did not undergo myomectomy [8]. However, observational and clinical experience appears to confirm that there can be benefits of surgery on any myoma which impinges into the uterine cavity regarding reproductive outcomes and live births.

There are multiple medical treatments for symptomatic myomas. Oral contraceptive pills are typically the first-line treatment for patients who are not interested in conceiving. Similarly, gonadotropin-releasing hormone agonists (GnRH-a) and more recently antagonists, available orally (Orilissa), are good options for patients who do not desire fertility [9]. Selective progesterone receptor modulators (SPRMs) (ulipristal acetate—UPA) were introduced as a recent alternative to GnRH-a treatment, especially in view of better tolerance to the drugs and the absence of negative impact on bone density. However, the impact of SPRMs on future fertility has not been well documented [10]; indeed, due to its potential serious side effects on the liver, the use of UPA has been limited following a review of the European Medicines Agency [11].

All current medical treatments typically have a short-term impact on the fibroids and their symptoms, and the latter quickly returns when treatment is discontinued. In addition, these drugs are generally contraindicated in patients who are pursing fertility treatment. Therefore, the definitive and best option for infertile patients remains surgical.

Historically, myomectomy was performed via an abdominal approach with a midline (vertical) or Pfannenstiel (transverse) incision. With advances in endoscopic surgical techniques and improved instrumentation as well as specialized gynecologic surgical training, less invasive surgical options have become the first line of treatment.

Intra-mural and subserosal myomas can be safely treated laparoscopically. This has multiple advantages over the classical abdominal approach. Most notably, a faster recovery, a lower risk of infections and bleeding, as well as a faster return to normal activity should be expected. More recently, when offered, robotic-assisted laparoscopic myomectomies have become an attractive option aimed at converting open surgeries to minimally invasive ones. However, it is worth noting that with experienced surgeons, there is no evidence showing a clear advantage of robotic over standard laparoscopic myomectomies on subsequent symptom improvement or fertility. As a matter of fact, the same Cochrane review [7] showed that current

evidence does not indicate that a superior method occurs among approaches involving laparoscopy, laparotomy, or different electrosurgical system as far as rates of live birth, clinical pregnancy, or miscarriage are concerned.

Regardless of whether laparoscopy is performed with or without robotic assistance, large myomas can be removed from the abdomen by introducing a power morcellator through a 10- or 15-mm port. Using the morcellator, large myomas are cut into small pieces in the abdomen to facilitate removal and the risk of seeding macro or microscopic fibroid fragments is decreased. Despite the low risk of unexpected leiomyosarcoma, from 1 in 770 to 1 in 10,000 myoma specimens [12], the FDA issued a warning contraindicating the use of power morcellation for the removal of uterine tissue containing myoma [12].

Therefore, many surgeons have reverted to the original abdominal approach despite the known higher risk of morbidity. Alternately, some surgeons elected to perform laparoscopic-assisted ultra-mini-laparotomy. The technique involves performing all or most of the myomectomy laparoscopically. Prior to removing the specimen from the abdomen, a 3–5 cm Pfannenstiel incision is made, and the specimens are delivered through the mini-laparotomy. This can be done either directly or by placing the myoma in an endoscopic bag. Depending on the location and extent of the incision, the uterine incision can then be closed laparoscopically or through the same mini-incision. With this technique, patients still have the benefit of lower complication rates as well as a faster recovery and are usually discharged home within a day. Most recently, the FDA allows power morcellation only with the use of a tissue containment system (Pneumoliner), allowing some surgeons who have access to the system to resume performing laparoscopic and robotic myomectomies.

Patients with submucosal myomas should first be offered a hysteroscopic approach, even in the presence of a larger sized myoma. With appropriate counseling and expertise, the submucous resection (SMR) can be performed safely with a rapid recovery. In addition, the time interval required before attempting conception is typically shorter after hysteroscopic SMR when compared to the abdominal approach, even when the SMR is performed over multiple sessions as described in the illustrated case here. Following any SMR, it is recommended that the cavity is re-evaluated, either with a midcycle ultrasound to confirm the presence of homogenous trilaminar endometrial stripe or with a saline infusion sonogram.

References

1. Baird DD, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. Am J Obstet Gynecol. 2003;188(1):100–7.
2. Stewart EA, Cookson CL, Gandolfo RA, Schulze-Rath R. Epidemiology of uterine fibroids: a systematic review. BJOG. 2017;124(10):1501–12.
3. Munro MG, Critchley HO, Broder MS, Fraser I, FIGO Working Group on Menstrual Disorders. FIGO classification system (PALM-COEIN) for causes of abnormal uterine bleeding in non-gravid women of reproductive age. Int J Gynaecol Obstet. 2011;113(1):3–13.

4. Practice Committee of the American Society for Reproductive Medicine. Removal of myomas in asymptomatic patients to improve fertility and/or reduce miscarriage rate: a guideline. Fertil Steril. 2017;108(3):416–25.

5. Pritts EA, Parker WH, Olive DL. Fibroids and infertility: an updated systematic review of the evidence. Fertil Steril. 2009;91(4):1215–23.

6. Parazzini F, Tozzi L, Bianchi S. Pregnancy outcome and uterine fibroids. Best Pract Res Clin Obstet Gynaecol. 2016;34:74–84.

7. Metwally M, Raybould G, Cheong YC, Horne AW. Surgical treatment of fibroids for subfertility. Cochrane Database Syst Rev. 2020;1(1):CD003857.

8. Casini ML, Rossi F, Agostini R, Unfer V. Effects of the position of fibroids on fertility. Gynecol Endocrinol. 2006;22(2):106–9.

9. Schlaff WD, Ackerman RT, Al-Hendy A, Archer DF, Barnhart KT, Bradley LD. Elagolix for heavy menstrual bleeding in women with uterine fibroids. N Engl J Med. 2020;382(4):328–40.

10. Donnez J, Dolmans MM. Hormone therapy for intramural myoma-related infertility from ulipristal acetate to GnRH antagonist: a review. Reprod BioMed Online. 2020;41(3):431–42.

11. Donnez J, Arriagada P, Marciniak M, Larrey D. Liver safety parameters of ulipristal acetate for the treatment of uterine fibroids: a comprehensive review of the clinical development program. Expert Opin Drug Saf. 2018;17(12):1225–32.

12. ACOG Committee. Uterine morcellation for presumed leiomyomas. ACOG Committee Opinion No. 822. Obstet Gynecol. 2021;137(3):e63–74.

Chapter 17
Ovarian Cysts

Hey-Joo Kang

Case

A 31-year-old G0 presented to the ER with acute right lower abdominal pain. She reported a 2-month history of vague right lower quadrant fullness. The pain increased with physical exertion, but usually abated spontaneously. For the past 2 months, she was able to continue her daily functions as an art curator, exercise 2–3 times per week, and had a normal appetite. Menses were regular and monthly, with a normal flow, and her annual gynecologic exams and pap smears have been normal. She had been on oral contraceptive pills for the past 8 years and discontinued them 6 months ago when she and her husband decided to start trying to conceive.

Over the course of the past 1–2 days, the pain became more frequent and intense. She described the pain to be sharp and intermittent, with the nausea and vomiting occurring in the moments the pain was most intense. Attributing the symptoms to a take-out dinner she ate the night before, she decided to go to bed early and wait the customary 24 h for her symptoms to resolve. In the morning the pain became worse. On her way to the emergency room, she had difficulty walking, requiring to stop frequently until her husband hailed a taxi to take them to the door of the emergency room.

Vitals signs showed a temperature of 98.7, heart rate of 128 bpm, and BP of 140/70. Physical examination showed she was in acute distress and diaphoretic. Examination of her abdomen revealed guarding but no rebound. Her urine pregnancy test was negative. Her pelvic exam was notable for a right adnexal fullness and she was very uncomfortable on a bimanual exam. Her white blood cell count was mildly elevated, but her hemoglobin count and chemistries were normal. A pelvic sonogram and CT scan were ordered. The pelvic sonogram

H.-J. Kang (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: hek9004@med.cornell.edu

The first responsibility of the clinician is to assess if the cyst is likely benign or malignant, although admittedly there is no absolute way to know without tissue pathology. Pelvic ultrasound uses gray scale to determine solid versus fluid and is highly effective at guiding the initial management of an ovarian cyst. Gray scale is a technique in which the ultrasound wave is depicted as a pixelated shade of gray; the darker the image, the longer the reflected wave took to return to the crystal. As such, fluid-filled structures appear black and solid structures appear light gray to give the sonographer a two-dimensional view of the anatomy [1]. Doppler evaluation uses the change in frequency that results from sound waves reflecting off moving particles, providing information about arterial and venous blood flow to certain areas of interest. Features of a simple cyst are a small size (<3 cm), a round/oval shape, uniformly dark (anechoic), thin walls, and absence of internal flow with color doppler imaging. Based on the 2019 Society of Radiologists consensus after review of 570 asymptomatic ovarian cysts [2], in premenopausal women, simple cysts up to 3 cm are considered normal. Simple cysts >3 cm but <5 cm should be described but do not require follow-up imaging, and >5 cm should have follow-up imaging to determine any change in size or spontaneous resolution.

Mature cystic teratomas (dermoid) are composed of differentiated elements from all three cell layers (ectodermal, mesodermal, and endodermal) and compose 95% of all ovarian teratomas. Dermoid cysts form by the failure of meiosis II or from a premeiotic cell in which meiosis I has failed [3]. They are almost always benign, but in 0.2–2% of cases, the components of a mature cystic teratoma can undergo malignant degeneration—the most common being squamous cell carcinoma arising from the ectoderm [4]. Risk factors for malignancy in a dermoid cyst is age over 45, tumor greater than 10 cm, rapid growth, and increased doppler flow to the internal part of the cyst [5].

Most women with dermoid cysts are asymptomatic despite growth to large (>5 cm) sizes, and the cysts are usually found incidentally on exam or ultrasound. Ovarian torsion is seen with a fair degree of frequency due to the weight of the cyst contents; however, rupture of the thick-walled cyst is uncommon. Case series of expectant management of dermoid cysts estimated the incidence of torsion at 3.5–11% [6]. Chemical peritonitis can result from the rare event of a rupture, leading to the formation of pelvic adhesions. The latter can be minimized by aggressive irrigation during surgery to remove the contents of the cyst spilled into the peritoneal cavity. Diagnosis of dermoid cysts by ultrasound shows a characteristic appearance with a heterogenous internal structure and a bright focus within the cyst with a reported specificity of 98–100% [7]. Treatment is pre-emptive ovarian cystectomy to allow for a tissue diagnosis while avoiding an emergency surgical intervention for torsion or rupture, versus expectant management. For women who have completed childbearing, unilateral oophorectomy is also a reasonable option. Dermoid cysts are bilateral in 10–20% of cases but rarely recur within the same ovary after cystectomy.

The prevalence of endometriosis in reproductive aged women is 5–10% and, when present, can lead to ovarian endometriomas formed by active endometrial tissue within the ovary [8]. It is estrogen dependent for growth. If symptomatic,

definitive surgical removal to establish pathology, prevent further growth and immediate relief of symptoms is an appealing option to many women. A less invasive approach is hormonal treatment in the form of progesterone IUDs, oral contraceptive pills, progesterone-only pills, or GnRH agonists.

For the asymptomatic women of reproductive age with a presumed endometrioma or those who desire fertility, watchful waiting versus surgical removal is the discussion initiated at the time of diagnosis. Risks of watchful waiting are lack of pathology (potential miss of a more sinister diagnosis), torsion, and rupture of the cyst. The larger the cyst (>4 cm), the more surgical intervention is appropriate to avoid rupture and torsion. For women who desire fertility, expectant management may allow for growth of the cyst and reduction of ovarian reserve. However, especially in women over 35 who are infertile, surgical management of a cystectomy may potentially impact upon the normal part of the ovary thus decreasing their ovarian reserve. In vitro fertilization (IVF) may be a wiser path to follow in these women. Surgical treatment prior to assisted reproductive therapy does not improve pregnancy outcomes [9]—the only exceptions being when large cysts hinder access to retrieve eggs and reduce the number of embryos obtained for IVF (refer to the chapter Endometriosis and Infertility).

As with all ovarian cysts, surgical treatment is the mainstay of definitively making a diagnosis to confirm they are benign. However, a less invasive approach is often prudent, especially in cysts <3 cm, and is appropriate when considering the age of the patient, desire for fertility, and any concerning sonographic features of the cyst. Medical management of endometrioma prior to surgery in young women or those at higher risk for surgery due to medical comorbidities should be considered as first-line treatment. Especially in women over 35 when fertility and egg counts are already reduced, surgery (cystectomy or oophorectomy) can risk further diminishment of ovarian reserve.

In conclusion, careful counseling on the risks and benefits of different management approaches on ovarian cysts is required in order to balance between the needs to establish pathology, alleviate symptoms, and prevent future complications such as torsion, and the concerns on a procedure requiring general anesthesia and potentially compromising ovarian reserve.

References

1. Utrilla-Layna J, Alcázar JL, Aubá M, Laparte C, Olartecoechea B, Errasti T, et al. Performance of three-dimensional power Doppler angiography as third-step assessment in differential diagnosis of adnexal masses. Ultrasound Obstet Gynecol. 2015;45(5):613–7.
2. Levine D, Brown DL, Andreotti RF, Benacerraf B, Benson CB, Brewster WR, et al. Management of asymptomatic ovarian and other adnexal cysts imaged at US Society of Radiologists in Ultrasound consensus conference statement. Ultrasound Q. 2010;26(3):121–31.
3. Caspi B, Lerner-Geva L, Dahan M, Chetrit A, Modan B, Hagay Z, et al. A possible genetic factor in the pathogenesis of ovarian dermoid cysts. Gynecol Obstet Investig. 2003;56(4):203–6.

4. Westhoff C, Pike M, Vessey M. Benign ovarian teratomas: a population-based case-control study. Br J Cancer. 1988;58(1):93–8.
5. Comerci JT Jr, Licciardi F, Bergh PA, Gregori C, Breen JL. Mature cystic teratoma: a clinicopathologic evaluation of 517 cases and review of the literature. Obstet Gynecol. 1994;84(1):22–8.
6. Hoo WL, Yazbek J, Holland T, Mavrelos D, Tong EN, Jurkovic D. Expectant management of ultrasonically diagnosed ovarian dermoid cysts: is it possible to predict outcome? Ultrasound Obstet Gynecol. 2010;36(2):253–40.
7. Tongsong T, Luewan S, Phadungkiatwattana P, Neeyalavira V, Wanapirak C, Khunamornpong S, et al. Pattern recognition using transabdominal ultrasound to diagnose ovarian mature cystic teratoma. Int J Gynaecol Obstet. 2008;103(2):99–104.
8. Nowak-Psiorz I, Ciecwiez SM, Brodowska A, Starczewski A. Treatment of ovarian endometrial cysts in the context of recurrence and fertility. Adv Clin Exp Med. 2019;28(3):407–13.
9. Tsoumpou I, Muglu J, Gelbaya TA, Nardo LG. The effect of surgical treatment for endometrioma on in vitro fertilization outcomes: a systematic review and meta-analysis. Fertil Steril. 2009;92(1):75–87.

Chapter 18
Decreased Ovarian Reserve

Isaac Kligman

Case

A 42-year-old nulligravid woman and her 43-year-old husband had been trying to conceive for the past 14 months. She states that for the past year her menstrual cycle intervals have shortened from 28 to 25 days and cycles sometimes occurred irregularly. She denies galactorrhea or hirsutism. Prior to their presentation to our Center, both hysterosalpingogram (HSG) and semen analysis were shown to be normal. She underwent two medicated (clomiphene citrate) intrauterine insemination (IUI) cycles without success.

Her past medical and surgical history were unremarkable. She gives a history of HPV infection although her PAP smears have been reported as normal. Menarche occurred at 11 years of age, with menstrual cycle interval of 28 days with 3–5 days of normal amount of bleeding in the past. She denies having dysmenorrhea or dyspareunia. She describes a coital frequency of about twice a week. She is an occasional smoker and consumes alcohol socially. She had no known drug allergies and denied using any medications regularly. Family history revealed a maternal aunt with colon cancer. Her 43-year-old husband has a 7-year-old son from a prior marriage, conceived without difficulty.

On physical exam, there was no focal abnormality noted. Pelvic exam revealed a normal size, anteverted uterus without palpable adnexal masses. Transvaginal ultrasound exam revealed four antral follicles on the right ovary and three antral follicles on the left ovary. Additional lab work included a normal thyroid profile, normal prolactin level, an AMH level of 0.6 ng/mL, and a day 2 FSH level of 13.8 IU/L.

With the diagnosis of primary infertility due to advanced maternal age and decreased ovarian reserve (DOR), the couple was advised to start IVF treatment.

I. Kligman (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: ikligman@med.cornell.edu

The stimulation protocol utilized Estradiol patches started in the mid-late luteal phase of the prior menstrual cycle. Once menstruation occurred, 300 IU of FSH, 150 IU of hMG began daily. GnRH antagonist was added later to prevent premature ovulation before retrieval. Six eggs were retrieved, of which five were mature. Four fertilized with ICSI and three embryos were transferred on day 3 at the 8, 7, and 6 cells stages; the fourth embryo arrested on day 2 of development. The patient conceived and delivered a healthy baby girl at term via Cesarean section.

Discussion

The ovaries contain a finite and decreasing number of oocytes throughout a woman's lifespan; the greatest number is found at 20 weeks of intrauterine life at about 7–8 million in both ovaries which decreases to about 500,000 at the time of menarche. With each menstrual cycle, a cohort of follicles is recruited, from which a dominant follicle will be selected to ovulate while the others will undergo atresia. This phenomenon results in a dramatic decrease in oocyte numbers throughout a woman's reproductive life. The total number of oocytes remaining in a woman's ovaries at a given time is known as her ovarian reserve which may have a significant impact on her fertility potential [1]. Oocyte quality, in addition to quantity, also seems to be affected by advanced female age [2]. Data from preimplantation genetic testing (PGT-A) performed at our Center has demonstrated an incidence of aneuploidy in 36% of embryos in women <30 years of age, 50% of embryos in women between 35 and 37 years of age, and 90% of embryos in women between 42 and 43 years of age. This decline of oocyte quality may be due to an impairment in the formation of the meiotic spindle or could be a reflection of a malfunction in the selection process thus allowing the development of follicles that otherwise would have become atretic. Thus, women with decreased ovarian reserve undergoing IVF will exhibit decreased responses to ovarian stimulation, reduced number of eggs retrieved, reduced number of viable embryos available for transfer or PGT testing, ultimately resulting in lower embryo implantation rates, increased spontaneous abortion rates, and decreased delivery rates.

Other than age, possible etiologies for poor ovarian reserve include genetic causes such as fragile X syndrome, anatomical causes such as the presence of ovarian cysts and endometriosis (especially in the presence of endometriomas), prior ovarian surgery, and history of exposure to chemotherapeutic agents and/or pelvic radiation. Less frequently some young women may present with DOR due to ovarian autoantibodies or lowered expression of granulosa cell FSH receptors.

Current social trends have led women to delay pregnancy into their mid-30s to even early forties. As postponement of pregnancy becomes more prevalent, an increase of age-related infertility due to DOR has been witnessed. Ovarian reserve testing should be performed in all women with infertility as it aids in prognostic counseling and helps to direct treatment options. For patients who require IVF,

ovarian reserve assessment will not only help determine their prognosis but will also aid in individualizing an appropriate ovarian stimulation protocol.

Several tests have been utilized to evaluate ovarian reserve. Basal FSH and estradiol (E2) levels should be measured on cycle day 2/3, whereas AMH level can be evaluated on any day of the menstrual cycle as long as oral contraceptives pills are not being used. Counting the number of antral follicles (AFC) on both ovaries on cycle day 2–4 by transvaginal ultrasound exam can also be considered [3]. Provocative tests such as the Clomiphene Citrate Challenge Test (CCCT) are no longer widely used.

Basal FSH level is a reflection of the remaining follicular pool. The rise in basal FSH level is due to decreased E2 and inhibin feedback to the pituitary by the diminished ovarian follicular pool. A basal FSH level of >12 IU/L is a sign of ovarian aging/decreased ovarian reserve. However, it should be noted that FSH levels have significant inter and intra-cycle variability which may limit their usefulness as a clinical tool. Usually when it is elevated, a repeat test in a subsequent cycle is recommended. In spite of its limitation, repeatedly elevated basal FSH levels have been associated with a decreased response to ovarian stimulation with gonadotropins and a lowered chance of success with IVF [4].

AMH is a glycoprotein hormone that belongs to the transforming growth factor-beta superfamily. During fetal life, it is produced by the testicular Sertoli cells of the male fetus and participates in the degeneration of Mullerian structures (oviducts, uterus, and upper part of the vagina). In adult women, it is produced by the granulosa cells of pre-antral and small antral follicles but not by dominant follicles. Since these follicles and their production of AMH are gonadotropin independent, levels of this hormone are relatively stable between and within menstrual cycles, which make it a very convenient ovarian reserve testing tool. AMH levels decrease with increasing woman's age and are strongly correlated with the remaining number of pre-antral and early antral follicles thus making this hormone a very useful marker of ovarian reserve. Several assays, however, have been developed to measure AMH levels; each IVF program should establish its own normative values, as careful interpretation of these results is essential. In our Program, the cut-off level of 0.9 ng/mL is used to designate low responders. It is important to underscore that AMH level is a good predictor of ovarian response to gonadotropin stimulation in IVF but not a good predictor for pregnancy outcome. Moreover, the use of AMH in women not seeking fertility is limited, since several studies have demonstrated that this test does not necessarily predict future fertility [5].

AFC is the sum of follicles observed by ultrasound in both ovaries in the early follicular phase (measured on days 2–4 of the menstrual cycle). Antral follicles are defined as follicles measuring between 2 and 10 mm in average diameter on a two-dimensional plane, measured with a transducer with a minimum frequency of 7 MHz. The total number of antral follicles has been clinically used to predict response to ovarian stimulation with gonadotropins. A low AFC of 3–6 is associated with poor response to ovarian stimulation during IVF. Furthermore, a low AFC of 3–4 is highly specific (73–99%) for predicting cycle cancelation or fewer than 3–4 oocytes retrieved. However, AFC does not accurately predict success to conceive [6].

Early diagnosis and more aggressive therapies in patients with DOR are critical to improve treatment outcome. Women who are 35 years of age or older and desire fertility should be encouraged to test their ovarian reserve and pursue a basic infertility evaluation after 6 months of attempting a pregnancy (instead of waiting for a year as in the general definition of infertility). Semen analysis, an HSG and a pelvic ultrasound examination should be performed. Treatment should be instituted promptly to increase the monthly fecundity rate and shorten the time to conception.

If DOR is diagnosed, couples who are under 38 years of age and without any prior treatment can consider starting controlled ovarian hyperstimulation (COH) with Clomiphene Citrate or gonadotropins combined with intrauterine insemination (IUI) for a few months. If not pregnant, IVF will be the next step. Older women should be encouraged to start IVF immediately. Selecting the appropriate stimulation protocol in these patients to yield the highest number of mature eggs and subsequently embryos that are available for transfer and/or preimplantation genetic testing (PGT) is of critical importance. Despite different stimulation strategies, no clear conclusion has been established on which protocol would be most ideal.

The most commonly used protocol entails starting ovarian stimulation on day 2 of the menstrual cycle with 450–600 units of gonadotropins per day after basal ultrasound exam and bloodwork are performed. Gonadotropin releasing hormone (GnRH) antagonist are added later to prevent premature LH surge or ovulation before retrieval. Other stimulation protocols can include the use of oral contraceptives or estrogen patches starting cycle 2 or mid-luteal phase respectively in the cycle prior to gonadotropin stimulation. GnRH agonist starting on cycle day 2 can be used in combination with gonadotropins to achieve a "flare" effect of endogenous gonadotropin release from the pituitary to augment stimulation. Later in the stimulation, once pituitary is downregulated, the agonist will serve to prevent a premature LH surge.

Mild or minimal stimulation is a protocol utilizing a minimum dosage of gonadotropins, alone or with oral medications such as clomiphene citrate, to aim at collecting a reduced number of oocytes. Compared to a conventional protocol, some investigators have reported that mild or minimal stimulation protocol might reduce embryo aneuploidy rate in patients with DOR [7]. However, the evidence collected so far did not support these findings [8].

Growth hormone (GH) has been employed by some clinicians as an adjuvant in patients with DOR. It is believed that GH has a synergistic effect with FSH in stimulating follicular growth via insulin-like growth factor 1 (IGF1) receptors present in the granulosa cells. The use of GH in patients with DOR was given momentum by a meta-analysis published in 2009 [9]. But subsequent studies and reviews suggested that there was substantial heterogeneity as well as potential for bias in most of the studies involving GH usage [10, 11]. Because the definitive day of starting GH during ovarian stimulation and its appropriate dosage are not well defined, along with its high cost, the enthusiasm for GH as an adjunct in patients with DOR has been somewhat tempered.

Other adjuvants such as DHEA and CoQ10 have been implicated in improving outcomes with patients with DOR. The use of these supplements was based on

animal studies that have demonstrated that androgens are involved in follicular stimulation at the pre-antral and antral stages. This approach, however, has not been supported by properly designed studies in humans.

Ultimately the decision of when to stop autologous IVF treatment is not straightforward for patients with DOR. When patients have repeated failure of responding to ovarian stimulation, fertilization, embryonic development, and implantation, it is reasonable to stop treatment and offer alternatives including oocyte donation which has a potential success rate in the order of 60–70%.

References

1. Broekmans FJ, Soules MR, Fauser BC, Broekmans FJ, et al. Ovarian aging: mechanisms and clinical consequences. Endocr Rev. 2009;30(5):465–93.
2. Greenwood Jaswa E, McCulloch CE, Simbulan R, Cedars MI, Rosen MP. Diminished ovarian reserve is associated with reduced euploid rates via preimplantation genetic testing for aneuploidy independently from age: evidence for concomitant reduction in oocyte quality with quantity. Fertil Steril. 2021;115(4):966–73.
3. Practice Committee of the American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: a committee opinion. Fertil Steril. 2015;103(3):e9–e17.
4. Ata B, Seyhan A, Seli E. Diminished ovarian reserve versus ovarian aging: overlaps and differences. Curr Opin Obstet Gynecol. 2019;31(3):139–47.
5. ACOG Committee Opinion No. 773: the use of antimüllerian hormone in women not seeking fertility care. Obstet Gynecol. 2019;133(4):274–8.
6. Tal R, Seifer DB. Ovarian reserve testing: a user's guide. Am J Obstet Gynecol. 2017;217(2):129–40.
7. Baart EB, Martini E, Eijkemans MJ, Van Opstal D, Beckers NG, Verhoeff A, 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.
8. Alviggi C, Conforti A. Mild/moderate versus full stimulation. Fertil Steril. 2022;117(4):664–8.
9. Kolibianakis EM, Venetis CA, Diedrich K, Tarlatzis BC, Grisinger G. Addition of growth hormone to gonadotropins in ovarian stimulation of poor responders treated by in-vitro fertilization: a systemic review and meta-analysis. Hum Reprod Update. 2009;15(6):613–22.
10. Duffy JM, Ahmad G, Mohiyiddeen L, Nardo LG, Watson A. Growth hormone for in vitro fertilization. Cochrane Database Syst Rev. 2010:CD000099.
11. Hart RJ. Stimulation for low responder patients: adjuvants during stimulation. Fertil Steril. 2022;117(4):669–73.

Chapter 19
Primary Ovarian Insufficiency

Rony Elias

Case

A 33-year-old nulligravid Middle Eastern patient presented for a second opinion with a history of infertility for more than 12 years. She reports that her menstrual cycles have become irregular with shorter intervals since 3 years ago. She did not complain of hot flashes, vaginal dryness, or any other symptoms. Her obstetrical history was significant for a first trimester miscarriage following a natural conception at age 24. There was no D&C performed. Her past medical and surgical history was positive for a hysteroscopic polypectomy 4 years ago. Her 34-year-old male partner is healthy.

Her initial infertility workup before she presented to our Center was significant for a very low anti-Mullerian hormone (AMH) level at 0.03 ng/mL. Semen analysis was normal. The couple was advised to undergo in vitro fertilization (IVF) immediately. They failed a total of 5 IVF cycles, all of which were associated with very low egg yields (1–3 eggs) despite the use of high doses of gonadotropins. In two cycles, the couple managed to have viable embryos to transfer but did not achieve a pregnancy.

The initial physical exam was unremarkable at our Center. A transvaginal ultrasound exam revealed 2–3 antral follicles in each ovary and the endometrial lining was very thin, consistent with low estrogen milieu. Her workup demonstrated a normal TSH level, a normal metabolic panel, and negative fragile X screening. Karyotype was 46, XX. No adrenal antibodies were detected. As expected, her FSH level was elevated at 74.6 mIU/mL with a low estradiol level of 26 pg/mL. A dual-energy X-ray absorptiometry scan (DEXA) showed normal bone density.

R. Elias (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: rta9002@med.cornell.edu

The patient was diagnosed with primary ovarian insufficiency (POI) and counseled about her overall prognosis of conception as well as general health. Despite a very low probability of success with her own eggs, the couple desired to undergo another IVF cycle, especially as she was young and had several ovarian follicles on ultrasound. A hysterosalpingogram (HSG) revealed a normal uterine cavity with bilateral hydrosalpinges. A laparoscopic bilateral salpingectomy was performed; the pathology was consistent with chronic salpingitis. The impact of hydrosalpinges on IVF success rates is discussed in Chap. 20 of this book.

Following surgery, she underwent an IVF cycle utilizing a luteal estrogen priming protocol combined with clomiphene citrate and low-dose gonadotropins. A total of three eggs were retrieved and two fertilized. A fresh transfer of both embryos, however, did not result in a pregnancy. The patient had to return home at that point and came back almost 2 years later. She reported that her menstrual cycles became even more irregular and unpredictable (once every 3–6 months). She was placed on oral contraception pill (OCP) by her local gynecologist. Her repeat FSH was 22 mIU/mL and her ovaries were devoid of any follicle on ultrasound. We advised her to stop OCP and start oral estrogens with ultrasound monitoring of any follicular growth and blood work once every 1–2 week. In addition, cyclic progesterone was used every 4–6 weeks to shed the endometrium. Interestingly, her FSH levels gradually decreased and a developing follicle was noted 4 months later. When the follicle spontaneously reached 14 mm size, a GnRH antagonist and 225 units of Menopur were started. Four days later, ovulation was triggered with human chorionic gonadotropin and the couple were counseled to proceed with oocyte retrieval. One mature egg was retrieved and fertilized. Since her endometrial lining was deemed to be suboptimal, the embryo was frozen at the pronuclear stage (day 1). Following menstruation, she was started on a programmed frozen embryo transfer cycle. Endometrial lining was prepared optimally. A 3-celled embryo was transferred after 2 days of progesterone administration. Her pregnancy test was subsequently positive and sonogram 3 weeks later confirmed the presence of a fetus with appropriate crown rump length and a normal fetal heartbeat of 140 beats per minute. Noninvasive prenatal testing at 11 weeks of gestation showed a normal female karyotype and she delivered a healthy baby girl at term. She was advised to restart OCP once she stopped breast feeding to reduce the risk of hypoestrogenism related osteoporosis.

Discussion

Primary ovarian insufficiency (POI) is a rare but clinically important condition where the depletion or dysfunction of ovarian follicles occurs before age 40; it is characterized by menopausal levels of FSH and absent or irregular menstrual cycles. POI has previously been referred to as premature menopause or primary ovarian failure. However, POI is the preferred term advocated by the National Institute of Health since ovarian function can occur intermittently, though unpredictably, in some of these patients. Up to 5–10% of women with POI even experience

spontaneous conception and delivery [1]. Spontaneous POI (sPOI) affects 1% of women before age 40, and 0.1% of women before age 30 [2].

Multiple etiologies exist for POI; these include genetic, autoimmune, iatrogenic (secondary to chemotherapy, radiation or surgical extirpation of the ovaries), and spontaneous presentation (sPOI). In 90% of women of sPOI who have a 46, XX karyotype, a specific underlying cause cannot be identified. A very small percentage of sPOI cases are due to autoimmunity against steroidogenic cells, a process that may affect both the ovarian and adrenal function [3].

A premutation in the Fragile X Mental Retardation 1 (FMR1) gene is responsible for an estimated 6% of all POI patients who exhibit a normal karyotype [4]. The FMR1 gene contains a polymorphic trinucleotide (CGG) repeat, normally present in <45 copies. A full mutation of the gene is defined by >200 CGG repeats and is the cause of fragile X syndrome, the most common heritable form of mental retardation.

The most common genetic cause of POI is gonadal dysgenesis with or without Turner syndrome stigmata. Turner syndrome affects 1 in 2500 girls. Abnormal karyotypes can be detected in 50% of adolescents and 13% of young women presenting with primary or secondary amenorrhea. Although this group of patients commonly show pubertal or growth delays, many are not recognized until they are evaluated for menstrual irregularities.

The diagnosis of POI requires a careful history, physical examination and laboratory assessment of serum FSH, estradiol (E2) and AMH. POI is confirmed only when FSH levels of ≥30–40 mIU/mL are found on 2 occasions at least 1 month apart. Since thyroid disorders and hyperprolactinemia can also manifest with irregular menstrual cycles, it is recommended that thyroid stimulating hormone (TSH) and prolactin (PRL) levels are checked. Upon confirmation of a POI diagnosis, further evaluation should include: a karyotype, fragile X (FMR1 premutation) assessment, serum anti-adrenal antibody measurement, and a pelvic ultrasound. If adrenal antibodies are detected, there is a 50% probability of these patients developing adrenal insufficiency (a life-threatening condition) [5]. It should be noted that POI may precede adrenal insufficiency by many years, thus requiring early monitoring to avoid future morbidity and mortality.

Management of POI requires a multidisciplinary approach. First and foremost, psychologic counseling should be offered because impaired self-esteem and emotional distress may occur in these young women upon learning the diagnosis. The most common terms that these patients use to describe how they feel are "shocked," "devastated," and "confused" [6]. They should be educated on the pathophysiology of the condition, risks of comorbidities and their management, the need for hormonal replacement therapy (HRT) and future fertility. Once POI is diagnosed, patients should be evaluated at least annually to assess efficacy of HRT, monitor thyroid and adrenal functions, and follow bone density with DEXA scans.

For patients who do not desire future fertility, and assuming there is no medical contradiction to hormone therapy, estrogen and progesterone replacement is recommended [7]. This will significantly reduce the risk of osteoporosis and cardiovascular disease while vaginal atrophy, sexual dysfunctional, and overall quality of life

can also be improved. Estrogen replacement can be given in the form of vaginal ring (100 μg daily), transdermal patches (100 μg daily), or oral estradiol (2 mg daily). Since most of these patients are young with intact uteri, cyclic progestins should be given for 10–12 days per month to reduce the risk of endometrial hyperplasia and cancer. This can either be in the form of oral micronized progesterone 200 mg daily for 12 days monthly or medroxyprogesterone acetate 10 mg daily for 10–12 days. Alternatively, many patients prefer to take combined oral contraceptive pills. It is important to inform patients that all of the above regimens can be associated with occasional ovulation if follicles remain in the ovaries. Hormonal replacement can be continued until the average age of natural menopause is reached.

For patients who desire fertility, treatment is more challenging. Initially patients need to be cleared for pregnancy by ruling out and treating any associated conditions such as thyroid or adrenal disorders. Patients with Turner syndrome who wish to conceive should undergo cardiac imaging and obtain clearance from a cardiologist and maternal fetal medicine specialist for conceiving.

Nearly 60–70% of women with POI have ovarian follicles and often exhibit intermittent ovarian function that in some instances persists for decades. This diagnosis should be considered separate and distinct from ovarian failure. Typically women with POI exhibit tonic serum LH elevation which may cause premature luteinization of growing antral follicles thus reducing the probability of spontaneous ovulation and response to ovarian stimulation. Theoretically, treatment with physiologic hormone replacement therapy may enhance the ability of ovarian follicles to avoid premature luteinization and respond to an endogenous or exogenous stimulus from gonadotropins, undergo follicular maturation, and ovulate, as seen in our patient. Another mechanism by which estradiol may improve fertility rates is by suppressing chronically elevated FSH levels, which have been shown to downregulate granulosa cell FSH receptors. Therefore, estrogens allow for restoration of FSH receptors and enhance the response to exogenous gonadotropins in the remaining ovarian follicle pool.

If the patient is single without a committed partner, oocyte cryopreservation can be offered. However, depending on her age and ovarian reserve, the patient and her family should be counseled extensively about their expectations. Since egg yields will likely be very low, the need for multiple egg retrieval cycles should be explained. When there is a committed partner and pregnancy is not desired, IVF with embryo cryopreservation may be a more practical option.

For patients with the potential of producing more than a couple of eggs (based on FSH and AMH levels, and antral follicle count) we recommend IVF. We usually employ protocols involving estrogen priming combined with GnRH antagonist downregulation, along with a low or moderate dose of gonadotropins as a higher dosage of gonadotropins is not likely to recruit more follicles in these women. Depending on the embryo yield and quality, a fresh day 3 embryo transfer is usually performed. If FMR1 premutation is identified, genetic counseling and IVF with preimplantation genetic testing (PGT) should be offered. Oocyte donation and adoption remain as the last resorts if pregnancy does not occur with their own oocytes.

In conclusion, POI involves the trio of amenorrhea, elevated gonadotropins, and estrogen deficiency which are often associated with other long-term health consequences including vasomotor symptoms, vulvovaginal atrophy, psychological stress, increased risks of cardiovascular disease, decreased bone mineral density, and markedly reduced fertility. Our patient is one of the rare women with POI who was fortunate enough to have succeeded in the delivery of a healthy child through IVF. Like all women with POI, she must be followed up to ensure adequate treatment with hormonal replacement therapy aiming at supporting her gynecological, cardiovascular, bone, and sexual health.

References

1. Nelson LM. Clinical practice. Primary ovarian insufficiency. N Engl J Med. 2009;360:606–14.
2. Coulam CB, Adamson SC, Annegers LF. Incidence of premature ovarian failure. Obstet Gynecol. 1986;67:604–6.
3. Hoek A, Schoemaker J, Drexhage HA. Premature ovarian failure and ovarian autoimmunity. Endocr Rev. 1997;18:107–34.
4. Nelson LM, Covington SN, Rebar RW. An update: spontaneous premature ovarian failure is not an early menopause. Fertil Steril. 2005;83:1327–32.
5. Bakalov VK, Vanderhoof VH, Bondy CA, Nelson LM. Adrenal antibodies detect asymptomatic autoimmune adrenal insufficiency in young women with spontaneous premature ovarian failure. Hum Reprod. 2002;17:2096–100.
6. Alzubaidi NH, Chapin HL, Vanderhoof VH, Calis KA, Nelson LM. Meeting the needs of young women with secondary amenorrhea and spontaneous premature ovarian failure. Obstet Gynecol. 2002;99:720–5.
7. Sullivan SD, Sarrel PM, Nelson LM. Hormone replacement therapy in young women with primary ovarian insufficiency and early menopause. Fertil Steril. 2016;106:1588–99.

Chapter 20
Tubal Factor

Kolbe Hancock and Pak H. Chung

Case

A 34-year-old nulligravid female presents with 13 months of infertility. She reports regular monthly menstrual periods and having coital exposure with the aid of urinary LH monitoring. Gynecologic history is significant for an abnormal Pap smear 5 years before presentation, which was followed by a benign colposcopic biopsy. Subsequent Pap smears have been normal. More importantly, she states that she was found to have a positive cervical chlamydial culture 8 years ago with a previous partner, which was treated successfully with oral antibiotics. Both she and her 36-year-old husband have had no significant medical problems.

Her initial work up in our Center revealed an anti-Mullerian hormone (AMH) level of 3.2 ng/mL, and an antral follicular count (AFC) of 12 on cycle day 3. Her husband's semen analysis was normal. Hysterosalpingogram (HSG), however, revealed a normal uterine cavity and bilateral proximal tubal obstruction. Repeating the study with oral sedation was offered but the couple declined. Of note, she did not appear to be in much discomfort during the HSG. Mid-cycle transvaginal ultrasound demonstrated an 8 mm trilaminar endometrial lining without any evidence of ovarian cysts or an adnexal mass.

Management options were reviewed with the couple. A diagnostic laparoscopy could be considered to identify potential etiologies for proximal tubal obstruction. Her history of chlamydia and therefore possible tubal damage and/or pelvic/

K. Hancock
Reproductive Medicine and Ob/Gyn, Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

P. H. Chung (✉)
Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: pakchu@med.cornell.edu

peritubal adhesions could be the cause for the obstruction and hence her infertility. The other option was to procced with in vitro fertilization (IVF) which would bypass the fallopian tubes. The patient declined surgery and decided to proceed with IVF. Ovarian stimulation with gonadotropins yielded 15 oocytes of which 12 fertilized. Endometrial thickness was 10 mm. A single high-grade blastocyst was transferred. The remaining six blastocysts underwent preimplantation genetic testing for aneuploidy (PGT-A) and were frozen.

She did not become pregnant with the fresh embryo transfer. Of the six PGT-A tested blastocysts, three were euploid. She was subsequently scheduled for a natural cycle frozen embryo transfer (FET). On the day of her LH surge, however, ultrasound examination revealed a thin layer of free fluid in the endometrial cavity, a finding which persisted and was also visualized on the day of the scheduled transfer. Therefore FET was canceled.

Given all the findings up to this point of her treatment, a laparoscopy was subsequently performed which confirmed moderate peritubal and pelvic adhesions, and bilateral hydrosalpinges. Lysis of adhesions and bilateral salpingectomies were performed uneventfully. Follow-up evaluation of the cavity demonstrated a normal endometrial stripe with no fluid. The patient subsequently underwent a natural cycle FET, promptly conceived and delivered a healthy baby at term.

Discussion

Tubal disease is identified in approximately 25–35% of female factor infertility [1]. Tubal factor may range from subtle damage within the lumen of the fallopian tube, to outright blockage associated with hydrosalpinges and/or peritubal adhesions. The gold standard for evaluating tubal factor is the HSG. A hydrosalpinx, caused by a fluid buildup within a blocked fallopian tube, leads to the characteristic sausage-like appearance, easily discerned on HSG and even on ultrasound. More than half of the hydrosalpinges are due to salpingitis from PID (chlamydia, gonorrhea, rarely tuberculosis), but they may also result from prior history of ectopic pregnancy, generalized peritonitis, and pelvic inflammation of various etiologies (ruptured appendix is a notable example). However, one needs to recognize that tubal obstruction shown on HSG can sometimes be caused by tubal spasm of the interstitial region secondary to the irritation of the contrast on the tube [2]. Typically, if spasm is the etiology, patients may experience a higher degree of discomfort during the procedure. This may be relieved by sedatives allowing filling and spillage of dye through the fallopian tube.

Many variables need to be considered when counseling patients with tubal factor infertility. Chronological age of the female, ovarian reserve, presence of male factor, site and extent of the tubal disease, experience of the surgeon, and access to IVF are all factors to be discussed with the patient. Management of tubal factor infertility depends on the location of the pathology, i.e. proximal or distal, whether it is unilateral or bilateral, and the extent of the disease.

Presuming that all other factors are optimal, unilateral obstruction can be managed by expectant management, controlled ovarian hyperstimulation with or

without intrauterine insemination (IUI), surgery or IVF. Expectant management may involve ultrasound monitoring of ovulation in a natural cycle to determine if ovulation will occur on the side of the patent fallopian tube. If so, timed intercourse or intrauterine insemination (IUI) can be performed. Probability of conception when ovulation occurs on the blocked side is significantly lower. Superovulation using oral medications will improve the odds of having a mature follicle on the patent side. However, surgery or IVF are the only viable options in the setting of bilateral obstruction. Even with IVF where embryos are transferred to the uterine cavity, all patients with tubal factor should be counseled about a higher risk of having an ectopic pregnancy.

Proximal tubal obstruction, representing 10–25% of tubal diseases, can be treated with tubal cannulation under fluoroscopic guidance or by hysteroscopy with laparoscopic confirmation [3]. Tubal cannulation could relieve 85% of apparent obstructions although one-third of the initially reported cannulated tubes subsequently re-occluded. The treatment of choice for unilateral proximal obstruction has not been conclusively established. One study demonstrated that such patients had similar cumulative pregnancy rates after three cycles of controlled ovarian hyperstimulation and IUI when compared to patients with unexplained infertility [4].

Distal obstruction is usually associated with fimbrial phimosis, hydrosalpinx, and/or pelvic adhesions. Laparoscopic neosalpingostomy is carried out by opening an obstructed tube. There is fair evidence that laparoscopic fimbrioplasty or neosalpingostomy can be considered for treating distal obstruction (even with mild hydrosalpinx) in young women who have no other significant infertility factor. However, patients should be aware that pregnancy rates are directly correlated to the degree of tubal disease and are more favorable in patients with "good prognosis," defined in the literature as having limited filmy adhesions, mild tubal dilation <3 cm, thin pliable walls, and endosalpinx with preserved mucosal folds [5]. Specifically, the intrauterine pregnancy rate after surgery for mild hydrosalpinges ranges from 58 to 77%, while the ectopic pregnancy rate after the procedure is reported to be between 2 and 8%. For comparison, in patients with more severe disease, the pregnancy success rate after surgery ranges from 0 to 22% with an ectopic rate ranging from 0 to 17%. Postoperatively, re-occlusion/reappearance of hydrosalpinx may occur, necessitating salpingectomy later.

In our practice, tubal surgery is only offered to patients with optimal ovarian reserve, absence of other compounding factors for infertility, mild tubal disease without significant pelvic adhesions, and who are reluctant to proceed with or have no access to IVF. Otherwise, IVF which overcomes tubal blockage is our preferred treatment.

In the setting of severe tubal damage with hydrosalpinges, there have been numerous studies demonstrating that these dilated tubes can have a detrimental effect on IVF success rates. There are multiple theories regarding the negative physiologic effects of hydrosalpinges on fertility. These include mechanical disruption or flushing of the embryo in the uterine cavity due to back flow of hydrosalpinx fluid which is inflammatory in nature, increased endometrial peristalsis/contractions,

decreased endometrial receptivity, altered endometrial blood flow, and the direct embryotoxic effect of the hydrosalpinx fluid on the embryo or sperm itself [6].

A meta-analysis on the impact of hydrosalpinx on IVF success demonstrated that hydrosalpinges caused a 50% decrease in pregnancy, implantation, and delivery rates, and a twofold increase in the spontaneous abortion rate [7]. Specifically, it is thought that those with hydrosalpinges large enough to be visible on ultrasound might be more significantly affected. Several randomized controlled trials have shown that laparoscopic salpingectomy in women with hydrosalpinges achieved similar live birth rates as compared to women without hydrosalpinx [8]. Even in patients with unilateral hydrosalpinx, higher IVF pregnancy rates have been reported after unilateral salpingectomy. Therefore we generally recommend salpingectomy for patients with unilateral or bilateral hydrosalpinges before undergoing IVF.

Salpingectomy can be readily performed laparoscopically by sequentially coagulating and dividing the mesosalpinx, generally from the distal to proximal end of the fallopian tube. Upon reaching the proximal end, the tube is cauterized and divided as close to the cornua as possible. It should be noted that there had been reports suggesting that salpingectomy and tubal ligation are associated with subsequent diminished ovarian reserve; however the clinical significance of this observation has been debated. While it has been demonstrated that antral follicle count and blood flow to the ovary are reduced after laparoscopic salpingectomy performed for ectopic pregnancy [9], in one such study where IVF was performed before and after salpingectomy, no significant difference was found in the total dose or duration of gonadotropins administered, peak estradiol levels, number of oocytes retrieved or embryo quality between cycles or between the ovaries [10]. Regardless, during salpingectomy, great care should be exercised to resect as close to the tube as possible along the mesosalpinx in order to avoid compromising blood supply to the ovaries.

Proximal occlusion of the fallopian tube during laparoscopy can be an alternative to salpingectomy when severe pelvic adhesions preclude access to the entire tube for removal. Previously published randomized controlled trials demonstrated that proximal tubal occlusion was effective in restoring IVF pregnancy rates in women with hydrosalpinges [11]. In addition, it has not been shown that this technique had any negative impact on ovarian reserve [12]. The preferred method for occlusion, cautery or mechanical clips, is debated. A randomized controlled trial found that bipolar cautery had an adverse effect on ovarian volume and antral follicle counts while mechanical clips did not [13]. However, neither technique was associated with appreciable changes in day-3 FSH, estradiol, inhibin-B, or anti-Mullerian hormone levels. Another theoretical concern of proximally occluding the tube is that it may lead to increased swelling of the hydrosalpinx, as the fluid is precluded from draining into the uterus, which in turn may lead to increased incidence of pelvic pain and risk of torsion.

Ultrasound-guided aspiration of hydrosalpinges at the time of oocyte retrieval is a less invasive option. The literature supporting such an approach is sparse, with conflicting results in two small retrospective studies [14, 15]. A randomized controlled study comparing ultrasound-guided aspiration to a nontreated control group reported significantly higher clinical pregnancy rates of 31.3% with aspiration

versus 17.6% in untreated controls [16]. However, the potential spread of the hydrosalpinx fluid in the pelvis/abdomen after aspiration may increase risks of postoperative infection after oocyte retrieval. Therefore careful considerations need to exercised before this option is recommended.

Back to our patient, initial HSG (proximal tubal obstruction did not allow dye to enter and distend the tubes) and ultrasound examination did not readily demonstrate presence of hydrosalpinges. Detecting fluid in the uterine cavity during a mid-cycle ultrasound examination while she was monitored for a FET raised the suspicion of back flow of hydrosalpinx fluids. Indeed subsequent removal of the tubes appeared to improve the uterine environment and optimize implantation leading to a successful pregnancy outcome.

References

1. Practice Committee of the American Society for Reproductive Medicine. Role of tubal surgery in the era of assisted reproductive technology: a committee opinion. Fertil Steril. 2021;115(5):1143–50.
2. Thurmond AS, Machan LS, Maubon AJ, Rouanet JP, Hovsepian DM, Moore A, et al. A review of selective salpingography and fallopian tube catheterization. Radiographics. 2000;20(6):1759–68.
3. Honore GM, Holden AE, Schenken RS. Pathophysiology and management of proximal tubal blockage. Fertil Steril. 1995;71(5):785–95.
4. Farhi J, Ben-Haroush A, Lande Y, Fisch B. Role of treatment with ovarian stimulation and intrauterine insemination in women with unilateral tubal occlusion diagnosed with hysterosalpingography. Fertil Steril. 2007;88(2):396–400.
5. American Fertility Society. The American Fertility Society classifications of adnexal adhesions, distal tubal occlusion, tubal occlusion secondary to tubal ligation, tubal pregnancies, Mullerian anomalies and intrauterine adhesions. Fetil Steril. 1988;49(6):944–55.
6. Practice Committee of the American Society for Reproductive Medicine. Salpingectomy for hydrosalpinx prior to in vitro fertilization. Fertil Steril. 2008;90(5 Suppl):S66–8.
7. Camus E, Poncelet C, Goffinet F, Wainer B, Meriet F, Nisand I, et al. Pregnancy rates after in-vitro fertilization in cases of tubal infertility with and without hydrosalpinx: a meta-analysis of published comparative studies. Hum Reprod. 1999;14(5):1243–9.
8. Strandell A. Treatment of hydrosalpinx in the patient undergoing assisted reproduction. Curr Opin Obstet Gynecol. 2007;19(4):360–5.
9. Chan CC, Ng EH, Li CF, Ho PC. Impaired ovarian blood flow and reduced antral follicle count following laparoscopic salpingectomy for ectopic pregnancy. Hum Reprod. 2003;18(10):2175–80.
10. Dar P, Sachs GS, Strassburger D, Bukovsky I, Arieli S. Ovarian function before and after salpingectomy in artificial reproductive technology patients. Hum Reprod. 2000;15(1):142–4.
11. Stadtmauer LA, Riehl RM, Toma SK, Talbert LM. Cauterization of hydrosalpinges before in vitro fertilization is an effective surgical treatment associated with improved pregnancy rates. Am J Obstet Gynecol. 2000;183(2):367–71.
12. Nakagawa K, Ohgi S, Nakashima A, Horikawa T, Irahara M, Saito H. Laparoscopic proximal tubal division can preserve ovarian reserve for infertility patients with hydrosalpinges. J Obstet Gynaecol Res. 2008;34(6):1037–42.
13. Goynumer G, Kayabasoglu F, Aydogdu S, Wetherilt L. The effect of tubal sterilization through electrocoagulation on the ovarian reserve. Contraception. 2009;80(1):90–4.

14. Sowter MC, Akande VA, Williams JA, Hull MG. Is the outcome of in-vitro fertilization and embryo transfer treatment improved by spontaneous or surgical drainage of a hydrosalpinx? Hum Reprod. 1997;12(10):2147–50.
15. Van Voorhis BJ, Sparks AE, Syrop CH, Stovall DW. Ultrasound-guided aspiration of hydrosalpinges is associated with improved pregnancy and implantation rates after in-vitro fertilization cycles. Hum Reprod. 1998;13(3):736–9.
16. Hammadieh N, Coomarasamy A, Ola B, Papaioannou S, Afnan M, Sharif K. Ultrasound-guided hydrosalpinx aspiration during oocyte collection improves pregnancy outcome in IVF: a randomized controlled trial. Hum Reprod. 2008;23(5):1113–7.

Chapter 21
Endometrial Factor

Ashley Aluko and Joshua Stewart

Case

A 31-year-old G0 presents for a second opinion after 2 failed frozen embryo trans-fers. Her history was notable for polycystic ovary syndrome and irregular menses. Due to anovulatory infertility, she was initially treated with three cycles of letrozole ovulation induction and intrauterine insemination. None of these cycles resulted in a pregnancy, and the couple then pursued IVF. Her first IVF cycle yielded six euploid blastocysts, and her three subsequent medicated frozen embryo transfer cycles failed to result in a positive serum HCG. In all of her cycles, the endometrial lining was greater than 7 mm and had a trilaminar appearance prior to transfer.

The patient, who grew increasingly frustrated during her course of treatment, was searching for a rationale behind her implantation failures. Her medical history was significant for Crohn's disease, which was well-controlled with infliximab. She had undergone an exploratory laparotomy and ileocecal resection several years before and had no other previous surgeries. Her infertility evaluation had included a hysterosalpingogram, which demonstrated bilaterally normal, patent fallopian tubes and normal intrauterine contours. Her anti-Mullerian hormone level was 4.75 ng/mL. Her partner's semen analysis had normal parameters.

Before her consultation at our office, an endometrial receptivity analysis (ERA) performed during a mock medicated cycle revealed "receptive endometrium." We performed a diagnostic hysteroscopy followed with endometrial sampling. Pathology from the endometrial sampling was negative for syndecan-1 (CD138) immunohistochemistry staining.

A. Aluko · J. Stewart (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: jds9013@med.cornell.edu

Discussion

Successful implantation and placentation are important prerequisites for normal pregnancy [1]. The initial interaction between embryo and endometrium is complex, and even minor perturbations can derail this carefully choreographed process. It is, therefore, not surprising that the majority of spontaneously conceived embryos fail to implant. In fact, even with donor sperm insemination under optimal conditions, implantation rates rarely exceed 40% [2]. Although there is no standardized definition for recurrent implantation failure (RIF), it is generally diagnosed after "three consecutive IVF attempts, in which one to two embryos of high-grade quality are transferred in each cycle" [3].

During implantation, the dialogue between the embryo and uterine wall is bidirectional and requires both a competent embryo and receptive endometrium. Embryos that are morphologically abnormal or aneuploid often struggle to implant, however, implantation failure after the transfer of high-quality, euploid blastocysts is concerning for an endometrial etiology. These cases are challenging and require careful examination of the uterine cavity for pathology—leiomyomas, endometrial polyps, uterine synechiae, and retained products of conception. In addition, the functional quality of the endometrium should be assessed. Inadequate endometrial proliferation in response to estrogen exposure, insufficient progesterone effect during the luteal phase, and asynchrony between the embryo and endometrium can preclude normal implantation.

Intrauterine Pathology

Any distortion of normal uterine architecture has the potential to affect implantation by interfering with embryo transport, uterine peristalsis, and endometrial receptivity. In addition, space-occupying lesions diminish the surface area available for implantation. Leiomyomas, endometrial polyps, adenomyosis, uterine synechiae, and retained products of conception are examples of intrauterine pathology that can decrease the likelihood of achieving a successful pregnancy through IVF [4, 5]. Some of these structural abnormalities are discussed further in other chapters.

A variety of diagnostic modalities can identify intrauterine pathology. These include ultrasonography, three-dimensional (3D) ultrasonography, magnetic resonance imaging (MRI), hysterosalpingography (HSG), saline infusion sonohysterography (SIS), and hysteroscopy [6]. In our practice, less invasive methods such as ultrasonography, SIS, and HSG are often used as diagnostic tools. We perform an MRI when additional details for surgical planning are needed (ex: large leiomyomas) or when complex Mullerian anomalies are suspected. Hysteroscopy is reserved for patients with suspected intrauterine pathology requiring surgical correction or for diagnostic purposes in patients who have failed multiple IVF cycles.

Thin Lining

It is well established that increasing endometrial thickness prior to embryo transfer correlates with implantation success in IVF cycles [7, 8]. In two studies of women undergoing IVF, a trilaminar appearance was favorable, and a minimum endometrial thickness of 6–7 mm was necessary for pregnancy [9, 10]. Interestingly, successful IVF pregnancies have been reported in patients with endometrial linings as thin as 3.5–4 mm, however, this clearly is not the norm [11–13]. At our institution, we rarely transfer embryos when the endometrium is either thin (defined as less than 7 mm) or homogenous, as these appear to be suboptimal conditions for implantation.

Endometrial thinning can be a consequence of hormonal alterations from oral contraceptives, progestins, or clomiphene citrate or letrozole use [14, 15]. Furthermore, Asherman's syndrome can render the endometrium unresponsive to estrogen exposure, and should be suspected in any patient with a history of uterine instrumentation. The impaired endometrial development associated with Asherman's syndrome results not only from intrauterine scar formation, but also from myometrial fibrosis which reduces uterine perfusion and local delivery of hormones [14].

In patients who have a thin endometrium during the late follicular phase of their natural cycles, we opt for transfer of embryos in a medicated cycle, where both exogenous estrogen and progesterone are administered in order to prepare the uterine lining for implantation. If the endometrium remains refractory to exogenous estrogen in our standard medicated protocols, we typically increase the estradiol dose and consider alterations in the route of administration. If these measures fail to produce a desired effect, a diagnostic hysteroscopy should be considered. If this is unrevealing, we offer adjunctive treatment in select cases with vasoactive medications such as sildenafil, in an effort to facilitate endometrial perfusion by increasing uterine blood flow. Initial studies on vaginal sildenafil citrate by Sher et al. were able to demonstrate an improvement in endometrial thickness, as well as implantation and ongoing pregnancy rates in women with IVF failures attributed to poor endometrial development [16, 17]. Other vasoactive medications have also been studied, including low dose aspirin, pentoxifylline, and vitamin E [14, 15], however, the benefit of using these substances in RIF patients remains to be determined.

Endometrial Receptivity Analysis (ERA)

It is imperative that the embryo makes contact with the uterine wall during the fertile window of implantation, which occurs for only a few days during the luteal phase [1, 18]. The transition from proliferative to secretory endometrium, under the influence of rising progesterone levels, plays a critical role in establishing endometrial receptivity. A host of cytokines, growth factors, and lipids are also involved, which is reflected by the fact that certain genes are differentially expressed in receptive endometrium [1]. The Endometrial Receptivity Analysis (ERA) capitalizes on

this unique gene expression profile, or "transcriptomic signature," in order to determine the optimal timing for embryo transfer [19]. An ERA is typically performed through an endometrial biopsy obtained after 5 full days of progesterone exposure in a mock medicated frozen embryo transfer cycle [4]. When there is a "receptive" ERA result, such as in our patient's case, a frozen embryo transfer can be performed in a subsequent medicated cycle 5 days after initiation of progesterone. In patients with a "non-receptive" result, the day of transfer can be adjusted based on whether the endometrium was "pre-" or "post-receptive" on the day of biopsy.

ERA studies suggest that one in four women with recurrent implantation failure have a displaced window of implantation [20]. In this subset of patients, the ERA allows for individualization of embryo transfer protocols [21]. Although personalized embryo transfer in patients with recurrent implantation failure has been found to improve implantation and ongoing pregnancy rates in some patients [22, 23], there are studies that have failed to find any significant benefit to using the ERA [24]. In our practice, patients who have failed two or more frozen embryo transfer cycles with high-quality, euploid blastocysts are at least offered an ERA in an attempt to optimize timing of subsequent embryo transfers.

Chronic Endometritis

As part of her workup after two failed frozen embryo transfers, our patient had an endometrial biopsy to rule out chronic endometritis. Although chronic endometritis has long been considered a potential cause of reproductive failure, the extent to which this condition impacts fertility remains poorly understood [1]. Though often asymptomatic, chronic endometritis can present with abnormal uterine bleeding, pelvic discomfort, and leukorrhea [25]. Histologically, chronic endometritis is characterized by superficial stromal edema, increased stromal density, and plasma cell infiltration [26]. In vitro studies suggest that chronic endometritis reflects an aberrant immune response to microbial antigens, resulting in the excessive recruitment of lymphocytes to the endometrial stroma, which produce antibodies that negatively impact endometrial receptivity and implantation [27]. The abnormal inflammatory microenvironment of chronic endometritis has been associated with delayed differentiation of mid-secretory endometrium [28] and linked to altered endometrial gene expression during the implantation window [29].

Although there are no standardized criteria for diagnosing chronic endometritis, the presence of endometrial stromal plasma cells is both highly sensitive and specific [27]. Unfortunately, plasma cells can be difficult to detect using conventional hematoxylin and eosin (H&E) staining, even by skilled pathologists. Immunohistochemical staining for syndecan-1 (CD138), a plasma cell surface proteoglycan, can enhance identification of plasma cells within an endometrial biopsy specimen and is frequently utilized as an adjunct to improve the sensitivity of pathologic diagnosis [30, 31]. At our institution, we frequently request CD138 immunohistochemical staining in addition to H&E staining but recognize the many

limitations of this test. Notably, endometrial epithelial cells frequently express CD138, which can reduce specificity of CD138 staining and lead to overdiagnosis [27]. Furthermore, the results of CD138 testing can vary tremendously based on the clinical laboratory, their protocols for anti-CD138 antibody selection and dilution, and timing and method of endometrial sampling [27, 32].

Several treatment regimens have been studied in women with RIF and chronic endometritis. McQueen et al. reviewed 35 cases of chronic endometritis, most of which were treated with ofloxacin 400 mg and metronidazole 500 mg twice daily for 14 days. The test of cure was 94% after a single course of antibiotics, and the cure rate was 100% after two courses. Of note, nine patients in this study received an alternative antibiotic regimen—doxycycline, doxycycline, and metronidazole, or ciprofloxacin and metronidazole [33]. Doxycycline 100 mg twice daily for 14 days is another regimen commonly used in clinical practice, with proven efficacy in patients with RIF [34]

Conclusion

In conclusion, we described the case of a patient with RIF who failed three medicated frozen embryo transfer cycles with euploid blastocysts. Her ERA revealed a receptive endometrium and her endometrial biopsy did not show any evidence of chronic endometritis. She did not have any risk factors for Asherman's syndrome, and her endometrial thickness was adequate during all of her frozen embryo transfer cycles. We ultimately offered her a diagnostic hysteroscopy for further endometrial assessment, as well as additional frozen embryo transfer cycles at our center. We also discussed the option of surrogacy. As evidenced by this case, RIF can be challenging for clinicians and frustrating for patients. The American Society for Reproductive Medicine (ASRM) lists significant uterine anomaly and unidentified endometrial factor among indications for use of a gestational carrier [35]. Women with severe intrauterine adhesions not amenable to surgical repair or RIF despite transfer of high-quality embryos should also be counseled on the option of surrogacy.

References

1. Taylor HS, Fritz MA, Pal L, Seli E. Speroff's clinical gynecologic endocrinology and infertility. 9th ed. Alphen aan den Rijn: Wolters Kluwer; 2020.
2. Diedrich K, Fauser BCJM, Devroey P, Griesinger G, Evian Annual Reproduction (EVAR) Workshop Group. The role of the endometrium and embryo in human implantation. Hum Reprod Update. 2007;13(4):365–77.
3. Simon A, Laufer N. Repeated implantation failure: clinical approach. Fertil Steril. 2012;97(5):1039–43.
4. Kliman HJ, Frankfurter D. Clinical approach to recurrent implantation failure: evidence-based evaluation of the endometrium. Fertil Steril. 2019;111(4):618–28.

 5. Munro MG. Uterine polyps, adenomyosis, leiomyomas, and endometrial receptivity. Fertil Steril. 2019;111(4):629–40.
 6. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile female: a committee opinion. Fertil Steril. 2015;103(6):e44–50.
 7. Richter KS, Bugge KR, Bromer JG, Levy MJ. Relationship between endometrial thickness and embryo implantation, based on 1,294 cycles of in vitro fertilization with transfer of two blastocyst-stage embryos. Fertil Steril. 2007;87(1):53–9.
 8. Abdalla HI, Brooks AA, Johnson MR, Kirkland A, Thomas A, Studd JW. Endometrial thickness: a predictor of implantation in ovum recipients? Hum Reprod. 1994;9(2):363–5.
 9. Oliveira JB, Baruffi RL, Mauri AL, Petersen CG, Borges MC, Franco JG. Endometrial ultrasonography as a predictor of pregnancy in an in-vitro fertilization programme after ovarian stimulation and gonadotrophin-releasing hormone and gonadotrophins. Hum Reprod. 1997;12(11):2515–8.
10. Gonen Y, Casper RF. Prediction of implantation by the sonographic appearance of the endometrium during controlled ovarian stimulation for in vitro fertilization (IVF). J In Vitro Fert Embryo Transf. 1990;7(3):146–52.
11. Cruz F, Bellver J. Live birth after embryo transfer in an unresponsive thin endometrium. Gynecol Endocrinol. 2014;30(7):481–4.
12. Sundström P. Establishment of a successful pregnancy following in-vitro fertilization with an endometrial thickness of no more than 4 mm. Hum Reprod. 1998;13(6):1550–2.
13. Check JH, Cohen R. Live fetus following embryo transfer in a woman with diminished egg reserve whose maximal endometrial thickness was less than 4 mm. Clin Exp Obstet Gynecol. 2011;38(4):330–2.
14. Senturk LM, Erel CT. Thin endometrium in assisted reproductive technology. Curr Opin Obstet Gynecol. 2008;20(3):221–8.
15. Lebovitz O, Orvieto R. Treating patients with "thin" endometrium - an ongoing challenge. Gynecol Endocrinol. 2014;30(6):409–14.
16. Sher G, Fisch JD. Effect of vaginal sildenafil on the outcome of in vitro fertilization (IVF) after multiple IVF failures attributed to poor endometrial development. Fertil Steril. 2002;78(5):1073–6.
17. Sher G, Fisch JD. Vaginal sildenafil (Viagra): a preliminary report of a novel method to improve uterine artery blood flow and endometrial development in patients undergoing IVF. Hum Reprod. 2000;15(4):806–9.
18. Navot D, Scott RT, Droesch K, Veeck LL, Liu HC, Rosenwaks Z. The window of embryo transfer and the efficiency of human conception in vitro. Fertil Steril. 1991;55(1):114–8.
19. Díaz-Gimeno P, Horcajadas JA, Martínez-Conejero JA, et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. Fertil Steril. 2011;95(1):50–60.
20. Ruiz-Alonso M, Blesa D, Díaz-Gimeno P, et al. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Fertil Steril. 2013;100(3):818–24.
21. Ruiz-Alonso M, Galindo N, Pellicer A, Simón C. What a difference two days make: "personalized" embryo transfer (pET) paradigm: a case report and pilot study. Hum Reprod. 2014;29(6):1244–7.
22. Hashimoto T, Koizumi M, Doshida M, et al. Efficacy of the endometrial receptivity array for repeated implantation failure in Japan: a retrospective, two-centers study. Reprod Med Biol. 2017;16(3):290–6.
23. Patel JA, Patel AJ, Banker JM, Shah SI, Banker MR. Personalized embryo transfer helps in improving in vitro fertilization/ICSI outcomes in patients with recurrent implantation failure. J Hum Reprod Sci. 2019;12(1):59–66.
24. Bassil R, Casper R, Samara N, et al. Does the endometrial receptivity array really provide personalized embryo transfer? J Assist Reprod Genet. 2018;35(7):1301–5.

25. Kitaya K, Matsubayashi H, Yamaguchi K, et al. Chronic endometritis: potential cause of infertility and obstetric and neonatal complications. Am J Reprod Immunol. 2016;75(1):13–22.
26. Greenwood SM, Moran JJ. Chronic endometritis: morphologic and clinical observations. Obstet Gynecol. 1981;58(2):176–84.
27. Kitaya K, Takeuchi T, Mizuta S, Matsubayashi H, Ishikawa T. Endometritis: new time, new concepts. Fertil Steril. 2018;110(3):344–50.
28. Kitaya K, Yasuo T. Immunohistochemistrical and clinicopathological characterization of chronic endometritis. Am J Reprod Immunol. 2011;66(5):410–5.
29. Di Pietro C, Cicinelli E, Guglielmino MR, et al. Altered transcriptional regulation of cytokines, growth factors, and apoptotic proteins in the endometrium of infertile women with chronic endometritis. Am J Reprod Immunol. 2013;69(5):509–17.
30. Bayer-Garner IB, Korourian S. Plasma cells in chronic endometritis are easily identified when stained with syndecan-1. Mod Pathol. 2001;14(9):877–9.
31. Bayer-Garner IB, Nickell JA, Korourian S. Routine syndecan-1 immunohistochemistry aids in the diagnosis of chronic endometritis. Arch Pathol Lab Med. 2004;128(9):1000–3.
32. Song D, Feng X, Zhang Q, et al. Prevalence and confounders of chronic endometritis in premenopausal women with abnormal bleeding or reproductive failure. Reprod Biomed Online. 2018;36(1):78–83.
33. McQueen DB, Bernardi LA, Stephenson MD. Chronic endometritis in women with recurrent early pregnancy loss and/or fetal demise. Fertil Steril. 2014;101(4):1026–30.
34. Johnston-MacAnanny EB, Hartnett J, Engmann LL, Nulsen JC, Sanders MM, Benadiva CA. Chronic endometritis is a frequent finding in women with recurrent implantation failure after in vitro fertilization. Fertil Steril. 2010;93(2):437–41.
35. Practice Committee of the American Society for Reproductive Medicine and Practice Committee of the Society for Assisted Reproductive Technology. Recommendations for practices utilizing gestational carriers: a committee opinion. Fertil Steril. 2017;107(2):e3–10.

Chapter 22
Recurrent Pregnancy Loss

Steven Spandorfer

Case

A 37-year-old G3P0030 with a 1-year history of trying to conceive but with 3 consecutive miscarriages is referred for an evaluation. She is currently cycle day 11 and will not had unprotected intercourse until she felt that she "better understands the cause of her miscarriages".

All three miscarriages occurred at approximately 7–9 weeks of gestation. She did not have a D&C for any of the pregnancies and required no instrumentation in passing the pregnancies. She reports no difficulties in getting pregnant, usually conceiving in the first 1–2 months of trying. She has had no testing yet except for preconception panels for her and her partner that were discordant for genetic abnormalities. She has taken no medications other than prenatal vitamins as recommended by her obstetrician. She specifically denies any history of thyroid disease. She denies a family history of miscarriages or "blood clotting" disorders. She did feel "like a failure" and that she had "let her husband and her family down" with these miscarriages.

She reported menarche at age 12 and previously regular menses with 28-day cycles. Her GYN history is otherwise unremarkable. She was up to date on her routine health care maintenance. Her past medical history was not contributory. She had a surgical history significant for an uncomplicated tonsillectomy at age 7. Her family history was significant for hypothyroidism in her mother and coronary artery disease in her father. She did not have any family history of cancer. She is a non-smoker, denies illicit drug use, and consumes alcohol socially (1–2 glasses per week).

S. Spandorfer (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: sdspando@med.cornell.edu

Physical exam showed normal vital signs and a BMI of 21.0. Examination of the thyroid revealed no enlargement or masses. Pelvic exam revealed normal external female genitalia, well-estrogenized vaginal mucosa, and a normal appearing cervix. Uterus was of normal size and there were no adnexal masses on bimanual exam. Transvaginal ultrasound showed normal uterus with a 7.5 mm endometrial thickness with a suggestion of a uterine septum extending down to within 2/3 of the cavity. Bilateral ovaries were of normal size and morphology. Her antral follicle count was approximately 25. She stated that she was quite anxious and wanted "everything tested."

She underwent a workup that revealed the following. Laboratory evaluation returned with a TSH of 2.3 mIU/L and normal free T4. Her thyroid antibody screen was negative. Her karyotype was 46 XX and her partner was 46 XY. Her HbA1C level was normal. A 3D saline-infusion-sonogram revealed what appeared to be uterine septum extending into the lower uterine segment. For confirmation, an MRI of the pelvis with contrast was performed which showed a uterine septum measuring 3.4 cm consisting of fibrous material. There was a normal outer uterine contour and the diagnosis of partial uterine septum was made. She had a full thrombophilia panel done including anti-cardiolipin antibodies, lupus anticoagulant, beta-2 glycoprotein 1 antibodies as well as the following—screenings for mutations in Factor V Leiden, methylenetetrahydrofolate reductase, prothrombin gene, and levels of homocysteine, anti-thrombin III, homocysteine, protein C and protein S. She underwent an endometrial biopsy on cycle day 8 which showed normal proliferative endometrium and no evidence of chronic endometritis. She had serial luteal progesterone levels determined at day 5, 7, and 9 after her surge which were all considered normal. Her husband had a DNA fragmentation test on his sperm which was found to be normal.

She was diagnosed with having a partial uterine septum as the etiology of her miscarriages. She subsequently underwent a hysteroscopic evaluation and resection of her septum. She was subsequently evaluated with a 3D saline-infusion-sonogram which determined the uterine cavity was normal without evidence of intra-uterine adhesions or residual septum.

She then attempted to conceive and became pregnant within 3 months. At a 7-week transvaginal sonogram, she was found to have a CRL of 6 mm, but no fetal heart activity was detected. A D&C was performed and cytogenetic testing revealed a trisomy-21. After further consultation, given the emotional burden associated with multiple miscarriages, she would consider IVF with preimplantation genetic testing for aneuploidy (PGT-A) before attempting another conception.

Discussion

The definition of recurrent spontaneous abortions (rSAB) is the loss of two or more pregnancies consecutively [1, 2]. It should be noted that this does not include molar pregnancies or ectopic gestations. The true prevalence is not entirely established but

is approximately 1.5% of all pregnancies [2]. Recurrent pregnancy loss requires a careful evaluation of the couple and should be handled delicately as this is a very sensitive and emotional issue. It should be emphasized to the couple that approximately 50% of the time an etiology will not be found [2]. This is partially due to the fact that most miscarriages are related to chromosome abnormalities or other abnormalities of the embryo itself. In a study of over 2000 pregnancies with confirmation of fetal cardiac activity, when cytogenetic analysis was obtained, approximately 70% were found to be karyotypic abnormal [3]. In this large study, maternal age was the strongest characteristic associated with miscarriage. In women under the age of 35 years, after detection of cardiac activity, the pregnancy loss rate was only 5%. On the other hand, in women over 40 years of age, the pregnancy loss rate exceeded 20% even after the detection of cardiac activity. Finally, it is important to recognize, the workup is similar in parous or nulliparous patients with no significant differences in the etiologic cause in either group [4].

In performing the workup, testing can be broken into two major categories consisting of well-established causes of recurrent miscarriage, and controversial and potential causes of recurrent miscarriage.

Established causes of recurrent pregnancy loss include: uterine anatomic issues (uterine septum, intra-uterine adhesions and sub-mucus fibroids), anti-phospholipid antibodies (anti-cardiolipin antibody, lupus anticoagulant and beta-2 glycoprotein antibodies), maternal endocrinological diseases (thyroid and diabetes), and genetic (parental translocations, inversions or other abnormalities that may lead to losses in offspring) [1, 2].

The clinician should also discuss with the couple's lifestyle factors that may be contributing to the increased pregnancy loss. Tobacco should be avoided and alcohol consumption should be limited. The patient should be counseled that maternal obesity is associated with an increased risk of miscarriage. In a recent study of over 7000 IVF patients, obese patients were almost twice as likely to have a miscarriage as compared to normal weight women [5].

Genetic abnormalities account for most miscarriages, but usually within the fetus [3, 4]. Karyotypic analysis of products of conception is helpful, particularly when usual abnormalities are found implicating the cause of the loss is not parental. This helps minimize the anxieties of not understanding the etiology of the pregnancy loss. For the workup, parental karyotypes should be obtained in couples experiencing recurrent miscarriages as 2–5% of couples have translocations. This is approximately tenfold increase over what is found in the general population [4].

Anatomic evaluation of the uterus is important. Abnormalities can be found in almost 20% of patients undergoing an evaluation for rSAB [1, 2]. Not surprising, studies that have used only hysteroscopy found abnormalities in up to 30% of patients [6]. Some of these abnormalities found include small polyps and other questionably significant abnormalities and explain the differences in the prevalence of uterine abnormalities found between groups of investigators. The evaluation of the uterus can be accomplished by transvaginal sonogram, 2D or 3D saline-infusion-sonogram (SIS), hysterosalpingogram or MRI. Generally, a SIS or HSG is the gold standard for assuring the uterine cavity is normal. Uterine abnormalities that can be associated with rSAB include uterine

septum, submucosal fibroids and intra-uterine adhesions. Some studies have even suggested that larger endometrial polyps are associated with rSABs. Repair of these abnormalities can be accomplished by hysteroscopic surgery. Removal of intra-uterine adhesions requires careful postoperative attention which usually involves the use of estrogen therapy and placement of an intracavitary balloon or stent to minimize recurrence of adhesions. The most important surgery to correct intra-uterine adhesions is the first attempt. Recurrent adhesions are often associated with a significantly worse prognosis for future fertility.

Approximately 5–20% of rSAB patients will be diagnosed with anti-phospholipid antibodies [1, 2, 4]. The tests most associated with rSAB's are lupus anticoagulant, anti-cardiolipin antibody and beta-2 glycoprotein antibodies. The antibodies have a variety of detrimental impact on both the cytotrophoblast and syncytiotrophoblast which negatively impact the early pregnancy. The standard therapy for anti-phospholipid antibodies in patients with rSAB's is baby aspirin and heparin or Lovenox. Other inherited thrombophilia abnormalities and immunologic abnormalities have been evaluated as potential causes of rSAB and these etiologies remain controversial. These include testing for factor V Leiden mutation, prothrombin II gene mutation, MTHFR gene mutation, homocysteine levels, Protein C and S activity as well as anti-thrombin III activity. Future studies may elucidate the value of these tests. The factor V Leiden mutation has been reported to be found in approximately 7% of all rSAB patients. Treatment of this remains controversial. Testing for serum cytokines, cytokine genetic polymorphisms, HLA matching and Natural Killer cells are not recommended [2].

Endocrine factors may play a role in rSAB. An evaluation for diabetes and thyroid function is important. Normal TSH level is generally taken as 0.5–5 mIU/L. However, TSH levels above 2.5 and below 5.0 may be associated with rSAB. This is particularly true in the presence of thyroid antibodies. Treatment is simple, usually involving low-dose thyroid hormone replacement. Diabetes that is well controlled is not a risk factor for miscarriages, but uncontrolled diabetes is associated with rSAB. It is reasonable to test hemoglobin A1C. In Kutteh's sentinel paper on over 1000 patients with rSABs, 7% of patients had thyroid abnormalities, but <1% had abnormal glucose testing [4]. Some studies have suggested that normalizing prolactin levels may reduce miscarriage risk.

The male partner contribution toward rSAB has mostly involved genetic abnormalities. Lately, new evidence has suggested that increased sperm DNA fragmentation is associated with rSAB. When using the TUNEL analysis, an almost fourfold increase in miscarriages has been reported when DNA fragmentation is abnormal [7]. There are many different assays measuring DNA fragmentation and few of the relevant studies have been prospective and controlled, thus there is still great controversy surrounding the impact of DNA fragmentation and rSABs. Increased DNA fragmentation can be caused by medications or other male anatomic issues such as varicoceles. If the DNA fragmentation is high, urologic referral/evaluation is warranted [7, 8].

Microbial infections are often evaluated, but evidence implicating this as a cause of rSAB is overall weak. On the other hand, chronic endometritis has been associated with rSAB [9]. Chronic endometritis is diagnosed by pathologic examination of the

endometrium demonstrating evidence of plasma cells on H&E stain of the endometrium. Specific staining for CD-138 (also known as syndecan) has improved the capability of diagnosing chronic endometritis. Staining for CD-138 has an approximate fivefold improvement in the diagnosis of chronic endometritis. Patients with rSAB have approximately a threefold increase in the prevalence of chronic endometritis when undergoing hysteroscopy and sampling of the endometrium. In a retrospective study, patients with untreated chronic endometritis had a 2.5-fold increased risk of miscarriage compared to those with no chronic endometritis on biopsy.

It is important to recognize the psychologic toil that repetitive pregnancy loss has on the couple. This patient felt like a "failure" and, after four consecutive losses, is in the need of psychological counseling and help. It is paramount for the treating physician to recognize this and refer the couple appropriately.

In conclusion, this patient presenting with recurrent miscarriages was found to have a uterine septum which was resected. She subsequently miscarried again with cytogenetic testing revealing an abnormality. She then opted for PGT-A as a way to ensure a euploid pregnancy. Generally, the diagnosis and treatment for recurrent miscarriages is not very straight forward. While no exact etiology is found in about 50% of the time, many of the causes of rSAB are not well-established or even controversial. However, for many patients with rSAB the long-term prognosis is quite favorable but is mostly associated with maternal age. Proper counseling with the couple about these facts is critically important.

References

1. ESHRE Guideline Group on RPL, Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, et al. EHSRE guideline: recurrent pregnancy loss. Hum Reprod Open. 2018;2018(2):hoy004.
2. Practice Committee of American Society of Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril. 2012;98(5):1103–11.
3. Spandorfer SD, Davis OK, Barmat LI, Chung PH, Rosenwaks Z. Relationship between maternal age and aneuploidy in IVF pregnancy loss. Fertil Steril. 2004;81(5):1265–9.
4. Jaslow C, Carney JL, Kutteh WH. Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. Fertil Steril. 2010;93(4):1234–9.
5. Romanski PA, Bortoletto P, Magaoay B, Chung A, Rosenwaks Z, Spandorfer SD. Live birth outcomes in infertile patients with class III and class IV obesity following fresh embryo transfer. J Assist Reprod Genet. 2021;38(2):347–55.
6. Zolghadri J, Momtahan M, Aminian K, Ghaffarpasand F, Tavana Z. The value of hysteroscopy in diagnosis of chronic endometritis in patients with unexplained recurrent spontaneous abortion. Eur J Obstet Gynecol Reprod Biol. 2011;155(2):217–20.
7. Schlegel PN, Sigman M, Collura B, De Jonge CJ, Eisenberg ML, Lamb DJ, et al. Diagnosis and treatment of infertility in men: AUA/ASRM guideline part II. Fertil Steril. 2021;115(1):62–9.
8. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod. 2012;27(10):2908–17.
9. McQueen DB, Perfetto CO, Hazard FK, Lathi RB. Pregnancy outcomes in women with chronic endometritis and recurrent pregnancy loss. Fertil Steril. 2015;104(4):927–31.

Chapter 23
Evaluation of Male Infertility

Caroline Kang and James Kashanian

Case

A 29-year-old male with no significant past medical history presents for fertility evaluation. He is married to a 28-year-old healthy female and they have been trying to conceive for the past 18 months. Despite the use of an ovulation predictor kit and no lubrication during regular, unprotected intercourse, they have not been able to successfully achieve a pregnancy. He reports never trying to conceive with another partner, nor does he have knowledge of achieving a prior pregnancy. His wife has not been pregnant in the past. She has been fully evaluated by a reproductive endocrinologist and no abnormalities were noted in her evaluation.

He has no past medical history and does not take any medication currently. He does not consume any vitamins or dietary supplements either. His surgical history is notable for tonsillectomy and left orchiopexy as a child due to undescended testicle. He has had no other prior urologic issue nor does he have issues with erectile function or ejaculation. He denies any history of groin or testicular trauma, sexually transmitted infections, nor testicular or epididymal infection. He has no family history of fertility issues; he has one older sister and one younger sister, each of whom has one child. He works as a high school math teacher, has never used tobacco, and drinks occasionally.

Physical exam is largely unremarkable. Heart, lung, and thyroid exam were normal. Abdominal exam revealed no surgical incisions and normal bowel sounds. Penile exam demonstrated a circumcised phallus, orthotopic urethral meatus, no

C. Kang
Urology, Department of Urology, Weill Medical College of Cornell University,
New York, NY, USA

J. Kashanian (✉)
Urology and Reproductive Medicine, Male Sexual Health, Department of Urology,
Weill Medical College of Cornell University, New York, NY, USA
e-mail: jak9111@med.cornell.edu

skin abnormalities on the glans or shaft, and no tunica albuginea plaques. Scrotal exam demonstrated 12 mL testis on the right, 10 mL testis on the left. Both testicles descended within the scrotum and there was no testicular mass, tenderness, and any evidence of a varicocele. Vasa are palpable bilaterally.

Two semen analyses were obtained by masturbation, separated by 2 weeks in time. The first semen analysis demonstrated a volume of 2.5 mL, 0.2 million sperm/mL, 5% motile sperm, 3% normal morphology, and no leukocytes. His second semen analysis demonstrated a volume of 2.8 mL, 0.15 million sperm/mL, 3% motile sperm, 3% normal morphology, and no leukocytes. Karyotype was 46, XY and Y-chromosome microdeletion (YCMD) assay was negative. Total testosterone was 478 ng/dL and follicle-stimulating hormone (FSH) level was 4.9 IU/mL.

Given very low sperm count and motility, in vitro fertilization (IVF) was recommended. Patient was instructed to bank multiple vials of sperm. IVF in conjunction with intracytoplasmic sperm injection (ICSI) was performed. After the first round of IVF, his wife became pregnant and gave birth to a healthy baby girl.

Discussion

Infertility affects approximately 15% of couples worldwide, with approximately half of cases due to male factor. Recent guidelines were published by the American Urological Association (AUA) and the American Society for Reproductive Medicine (ASRM) regarding evaluation of the infertile male, which allows for physicians taking care of these patients evaluating these infertile men in an evidence-based manner [1].

History

Men with concern for infertility or abnormal semen analyses should undergo evaluation by a male reproductive health specialist. A comprehensive reproductive and sexual history should be obtained. The components of a comprehensive history for evaluation of male infertility are documented in Table 23.1.

Physical Exam

The physical exam during infertility workup should be focused but also comprehensive. Recent studies have shown that infertility may be the first sign of systemic disease present in an individual. First, general appearance should be

Table 23.1 Components of history during male infertility evaluation

Reproductive history	Duration of infertility
	Achieved prior pregnancy?
	Partner characteristics (age, gynecologic history, gynecologic evaluation, prior pregnancies, menstrual cycle)
	Use of contraception
	Use of ovulation kits/timed pregnancy
	Prior assisted reproduction
	Prior treatments for infertility
Developmental history	Timing of puberty
	Pattern of hair growth
	Childhood illnesses
	Undescended testis
Past medical history	History of cancer (i.e., testicular)
	Exposure to gonadotoxins (medications, chemotherapy, radiation, other environmental exposures)
	Steroid or exogenous testosterone use or exposure
	History of cystic fibrosis
	Prior surgery for infertility
Past surgical history	History of orchiopexy
	History of inguinal hernia repair
	History of radical pelvic surgery (i.e., radical prostatectomy, cystoprostatectomy, abdominoperineal resection)
	History of testicular surgery (i.e., radical orchiectomy, retroperitoneal lymph node dissection)
Sexual history	Erectile dysfunction
	Ejaculatory dysfunction
	Sexual desire
	Frequency of intercourse
	Use of lubricants
Family history	History of infertility
	History of genetic disorders
	History of abnormal vision or smell

appreciated and any congenital abnormalities, syndromic features, secondary sexual characteristics, and body habitus should be documented. Masculinization in terms of hair distribution and gynecomastia should also be noted. During the abdominal and inguinal survey, one should examine the areas for well-healed scars that may indicate prior surgical procedures. Next, one should perform a thorough penile and scrotal exam. The penis should be examined for appropriate development, circumcision status, and location of meatus (to determine if hypospadias or epispadias is/was present—which may indicate abnormal development in utero). Scrotal exam should include assessment of the spermatic cord, testis, and epididymis. Spermatic cord examination should note presence or

absence of varicocele, hernia, and whether the vas deferens is palpable bilaterally. Testicular exam should note size and consistency of the testes, as well as the presence of any intratesticular or extra-testicular masses. If necessary, Tanner stage should be assigned if incomplete maturation is present. Epididymal exam should note whether it is dilated, indurated, or flat, and presence of any epididymal cysts. Digital rectal exam in some cases can be performed to determine the presence of any prostatic cysts or dilated/enlarged seminal vesicles.

Semen Analysis

If not already done, any male undergoing infertility evaluation should undergo at least one semen analysis. Although abnormal semen parameters do not necessarily indicate sub- or infertility, drastically abnormal parameters, such as azoospermia (zero sperm count) provide an obvious reason for infertility. Besides total sperm count and concentration, other parameters examined include volume of ejaculate, pH, motility (total and progressive), vitality (provided as a percentage of viable sperm), morphology (provided as a percentage of sperm with normal shape), and leukocyte count [2].

Laboratory Testing

For infertile men, laboratory evaluation of FSH and testosterone levels should be performed. FSH level and testis size, along with history, can help differentiate non-obstructive azoospermia from obstructive azoospermia. The vast majority of azoospermic men with an FSH level less than 7.6 mIU/mL along with testis size (long axis) greater than 4.6 cm will have obstructive azoospermia, thus, a diagnostic testicular biopsy is not required for definitive diagnosis [3].

Testosterone is necessary for spermatogenesis to occur, and men with low testosterone levels may be oligozoospermic or azoospermic. Additionally, hypogonadism may result in impaired libido, erectile dysfunction, and ejaculatory function. Intratesticular testosterone is concentrated by androgen-binding protein, which is secreted by Sertoli cells, and intratesticular testosterone is estimated to be much higher than that found in the serum [4]. In men with low testosterone, who desire preservation of their fertility, selective estrogen receptor modulators (SERMs e.g., clomiphene citrate), aromatase inhibitors (e.g., anastrozole), and gonadotropins (e.g., human chorionic gonadotropins) can be administered.

Additional labs that may be obtained during infertility workup include luteinizing hormone, estradiol, and prolactin. These hormones play a role in the hypothalamic-pituitary-testis (HPT) axis and alterations in hormone levels can result in altered testicular function and spermatogenic failure.

Genetic Testing

For any male with primary infertility and azoospermia or severe oligozoospermia (total sperm count <5 million/mL), karyotype and Y-chromosome microdeletion (YCMD) testing should be performed [1]. Chromosomal anomalies, such as Klinefelter syndrome (most commonly with a karyotype of 47, XXY), may be the underlying etiology for infertility. It is not uncommon for some men to be first diagnosed with Klinefelter syndrome during their fertility evaluation as young adults.

Three distinct azoospermia factor regions (AZF) of the Y-chromosome responsible for spermatogenesis are termed AZFa, AZFb, and AZFc. Microdeletions in these regions can result in severe spermatogenic dysfunction. Each region encodes for various genes with varying importance in spermatogenesis, and deletions of the various AZF factors can be partial, complete or in combination. Microdeletions in these regions are not detectable by karyotype and instead require polymerase chain reaction for detection. The presence of Y-chromosome microdeletions carries important prognostic information. AZFc microdeletions are the most common (up to 80%), followed by AZFb (5%) and AZFa (4%) [5]. Men with AZFa and AZFb are unable to father biological children as no sperm has been documented to be surgically retrieved in the literature. In contrast, men with AZFc demonstrate a range of spermatogenic dysfunction varying from being able to naturally conceive children to being completely azoospermic. Azoospermic men with complete AZFc deletions who undergo microdissection testicular sperm extraction (microTESE) have reported sperm retrieval rates up to 67% [6].

In men with vasal agenesis or idiopathic obstructive azoospermia, Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) mutation carrier testing should be obtained [1]. In men with CBAVD, CFTR testing in the female partner should also be considered given the significant health implications of having offspring with cystic fibrosis. It is important to note that when obtaining CFTR mutation carrier testing that an extended panel of mutations, including the 5T allele, should be included to ensure no mutations are missed [1]. It is estimated that up to 80% of men with congenital bilateral absence of the vas deferens, and approximately 20% of men with congenital unilateral absence of the vas deference or idiopathic epididymal obstruction harbor CFTR gene mutations [7].

Adjunctive Tests

Adjunctive tests that can be performed include sperm DNA fragmentation (SDF) analysis and anti-sperm antibody (ASA) testing. However, current guidelines recommend that initial evaluation of infertile couples should not make use of these tests [1]. SDF analysis may be considered in the setting of unexplained infertility, recurrent pregnancy loss, recurrent intrauterine insemination (IUI) or IVF failure,

varicocele, or if specific risk factors (such as increased age, presence of systemic or genital infections, exposure to environmental toxins or ionizing radiation) are present [8].

ASA testing should be considered only if the presence of antibodies will affect the management of the patient. Detection of antibodies has been reported to be associated with vasal or epididymal obstruction, and prior testicular trauma or surgery. The presence of ASA may negatively affect some forms of assisted reproduction techniques (ART), such as intrauterine insemination (IUI), but these effects may be bypassed with other forms of ART like intracytoplasmic sperm injection (ICSI) [9].

Imaging

No routine imaging is recommended during the evaluation of infertile men [1]. However, some imaging studies may be considered in special circumstances. Scrotal ultrasound imaging can be performed when the body habitus or consistency of the scrotal contents precludes a thorough examination of the spermatic cord or it is unclear whether a varicocele is present. When obtaining a scrotal ultrasound for varicocele evaluation, the size of and flow in the paratesticular veins should be the focus. The ultrasonographer should measure these parameters with the patient in the standing and supine positions with and without Valsalva maneuvers. Reversal of flow with Valsalva maneuvers should be documented. Typically, varicoceles are present if the veins are greater than 2.5 mm in diameters, or if the veins are serpiginous in appearances, or there is reversal of flow.

Renal ultrasonography may be considered in patients with vasal agenesis to evaluate for renal abnormalities [1]. Because the male reproductive tract is derived from the Wolffian duct, during embryogenesis, anomalies in the reproductive tract can occur with concomitant anomalies in the urinary tract. In men with unilateral absence of the vas deferens, 26–75% may have ipsilateral renal anomalies (including renal agenesis), whereas approximately 10% of men with bilateral absence of the vas deferens have renal anomalies [10].

In conclusion, specific guidelines exist for the evaluation of the infertile male in an evidence-based manner. Our patient suffered from severe oligozoospermia as well as asthenospermia (low motility) and teratozoospermia (abnormal morphology). His underlying spermatogenic dysfunction was likely due to his history of cryptorchidism. Despite early fixation of the testes in the scrotum by orchiopexy, irreversible damage to the testes can occur resulting in sub- or infertility. A thorough history and physical as well as laboratory testing is important so that appropriate counseling and effective treatments can be provided to the patient.

References

1. Schlegel PN, Sigman M, Collura B, DeJonge CJ, Eisenberg ML, Lamb DJ, et al. Diagnosis and treatment of infertility in men: AUA/ASRM guideline part I. J Urol. 2021;205(1):36–43.
2. Cooper TG, Noonan E, von Eckardstein S, Auger J, Gordon Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16(3):231–45.
3. Schoor RA, Elhanbly S, Niederberger CS, Ross LS. The role of testicular biopsy in the modern management of male infertility. J Urol. 2002;167(1):197–200.
4. Smith LB, Walker WH. The regulation of spermatogenesis by androgens. Semin Cell Dev Biol. 2014;30:2–13.
5. Bansal SK, Jaiswal D, Gupta N, Singh K, Dada R, Sankhwar SN, et al. Gr/gr deletions on Y-chromosome correlate with male infertility: an original study, meta-analyses, and trial sequential analyses. Sci Rep. 2016;6:19798.
6. Stahl PJ, Masson P, Mielnik A, Marean MB, Schlegel PN, Paduch DA. A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia. Fertil Steril. 2010;94(5):1753–6.
7. Yu J, Chen Z, Ni Y, Li Z. CFTR mutations in men with congenital bilateral absence of the vas deferens (CBAVD): a systemic review and meta-analysis. Hum Reprod. 2011;27(1):25–35.
8. Panner Selvam MK, Agarwal A. A systematic review on sperm DNA fragmentation in male factor infertility: laboratory assessment. Arab J Urol. 2018;16(1):65–76.
9. Check JH, Hourani W, Check ML, Graziano V, Levin E. Effect of treating antibody-coated sperm with chymotrypsin on pregnancy rates following IUI as compared to outcome of IVF/ICSI. Arch Androl. 2004;50(2):93–5.
10. Schlegel PN, Shin D, Goldstein M. Urogenital anomalies in men with congenital absence of the vas deferens. J Urol. 1996;155(5):1644–8.

Chapter 24
Erectile Dysfunction

Caroline Kang and James Kashanian

Case

A 64-year-old male with hypertension and hyperlipidemia presented for evaluation of worsening erectile function. Over the past 5–6 years he noted a decrease in erectile function. Currently, with sexual or self-stimulation, his rigidity is 60% (70% being rigid enough for penetrative intercourse). Additionally, he has been unable to maintain his erection. He is married to a 63-year-old female, and they report being intimate once per week and he masturbates once per week. He lacks nocturnal and morning erections. He denies penile curvature or decreased sensation of orgasm, and ejaculate volume is unchanged. He also denies hematospermia or pain during ejaculation. Lastly, he denies any abnormalities in energy, libido, concentration, or mood.

He takes lisinopril and atorvastatin daily. He is not taking nitrates nor has he ever required nitrates for chest pain in the past. He has good exercise tolerance and walks 2 miles daily. His surgical history consists of knee replacement. He denies any urological complaints or trauma to the groin or pelvis. His family history is notable for hypertension in his father but negative for cardiovascular disease or stroke. He has one healthy 30-year-old daughter.

Physical exam showed normal vital signs, a BMI of 29.0, and a well-nourished male. He has normal body hair growth pattern. He has no gynecomastia. Genital exam revealed a circumcised penis, orthotopic urethral meatus, no glans or penile shaft lesions. Pubic hair distribution was normal. Scrotal exam was unremarkable

C. Kang
Urology, Department of Urology, Weill Medical College of Cornell University, New York, NY, USA

J. Kashanian (✉)
Urology and Reproductive Medicine, Male Sexual Health, Department of Urology, Weill Medical College of Cornell University, New York, NY, USA
e-mail: jak9111@med.cornell.edu

with testis size 18 cc and firm, bilaterally. Total testosterone was 485 ng/dL and HgbA1c was 5.7%.

He underwent penile doppler ultrasound in the clinic. He was administered 20 units of intracavernosal vasoactive agent to induce erection. At time of ultrasound, rigidity was 80%. His right cavernosal artery measured 0.82 mm in diameter and had a peak systolic velocity of 22.7 cm/s and an end diastolic velocity of 0 cm/s with a resistance index of 1.0. His left cavernosal artery measured 0.76 mm in diameter and had a peak systolic velocity of 23.8 cm/s and an end diastolic velocity of 0 cm/s with a resistance index of 1.0. There was no penile curvature or tunica albuginea plaque noted on exam or ultrasound. Findings on penile ultrasound were consistent with arterial insufficiency.

At this time, he was started on a phosphodiesterase-5 inhibitor (PDE5i), Viagra 100 mg, as needed for sexual activity. He was instructed to take the medication on an empty stomach, with an 8-h window for sexual activity. He was referred to cardiology for evaluation, as his penile arterial insufficiency could be an indicator of early cardiovascular disease. He was also instructed on proper lifestyle modifications, including diet and exercise, to improve his cardiovascular and erectile health.

Discussion

Erectile dysfunction (ED) is the inability to obtain and/or maintain an erection sufficient for satisfactory sexual relations. ED affects millions of men, with estimates of 50% of men over 40 years being affected by some degree of ED [1]. There are various etiologies that result in ED including vascular disorders, neurologic conditions, iatrogenic or medication-induced, endocrine dysfunction, and psychologic issues. Various disorders related to ED are shown in Table 24.1.

Table 24.1 Etiologies of ED

Etiology	Disorders
Vasculogenic	Hypertension, dyslipidemia, diabetes, obesity, tobacco use, coronary artery disease
Neurogenic	Spinal cord injury, multiple sclerosis, Parkinson's disease, lumbosacral disc disease, traumatic brain injury, stroke
Iatrogenic	Radical pelvic surgery (radical prostatectomy, radical cystectomy, abdominoperineal resection), pelvic radiation, medications
Medications	Thiazide diuretics, beta blockers, spironolactone, anti-psychotics, tricyclic antidepressants, selective serotonin reuptake inhibitors, benzodiazepines, phenytoin, digoxin, anti-androgens, 5-alpha reductase inhibitors, H2-antagonists, opiates
Endocrine dysfunction	Hypogonadism, hyperprolactinemia, thyroid dysfunction
Psychogenic	Stress, anxiety, depression

Pathophysiology

The mechanism by which erection occurs involves smooth muscle relaxation, which is regulated by adrenergic fibers, myogenic, and endothelium-derived factors (such as prostaglandin and endothelin). Non-adrenergic non-cholinergic (NANC) nerves release nitric oxide (NO) and parasympathetic nerves release acetylcholine, both actions that result in increased cyclic GMP (cGMP) concentration and smooth muscle relaxation. With smooth muscle relaxation, blood fills the corpora cavernosa, which in turn compresses the subtunical venules preventing venous outflow. cGMP is hydrolyzed by phosphodiesterase type 5 (PDE5), which contracts the smooth muscle and reverses this process, allowing for penile detumescence. ED can occur with the disruption of any of the steps in this process, including nerve dysfunction, decreased arterial inflow, or inelasticity of the smooth muscle of the penis so that venous outflow cannot be compressed.

Evaluation

Evaluation of ED requires a comprehensive history, including history of present illness, past medical history, past surgical history, medication history, social history, and family history, along with a focused physical exam [2]. Components of the history that are essential to capture include the onset of ED, persistence of the problem, maximal penile rigidity, sustainability of the erection, nocturnal erectile function, libido, ejaculatory function, and orgasm sensation. Another set of important questions centers around the patient and his partner's sexual dynamics, including frequency of sex and whether spontaneity is important. Questions of past medical history should examine for vascular or neurologic risk factors, diabetes, and coronary artery disease. Past surgical history should focus on any surgical procedures that may compromise penile neural innervation or blood flow (such as radical pelvic surgery). Medications being taken should be completely documented to ensure offending medications are not the etiology for ED. Social history should evaluate for illicit drug and tobacco use. Family history should be taken to determine if the patient is at risk for vascular or neurologic disorders that may contribute to ED. Components of the physical exam important to perform include general body habitus evaluation, evaluation for gynecomastia, testicular volume and consistency, penile stretch, and penile pathology evaluation (i.e., tunica albuginea plaque). In addition to a thorough history and physical examination, validated questionnaires are recommended to document the severity of the disease, to measure treatment efficacy, and to guide future treatment options [2].

Laboratory evaluation should be performed to aid in diagnosis of the etiology and for treatment optimization for ED. An early morning total testosterone level should be obtained [2]. Men with low testosterone levels should be counseled that ED treatments coupled with testosterone replacement may be more effective than

without testosterone therapy. Optional hormone levels that also can be obtained include free (or bioavailable) testosterone, luteinizing hormone, estradiol, and prolactin levels. A screening hemoglobin A1c may be obtained in patients with clinical suspicion of pre-diabetes or diabetes.

In men with a known etiology for ED, empiric treatment with a PDE5-inhibitor (PDE5i) can be initiated [2]. However, if further evaluation of the vasculature of the penis is necessary, penile doppler ultrasound (PDUS) assessment can be performed in the clinic. The integrity of the penile vasculature is evaluated by measuring the change in the penile arterial diameter and flow velocity before and after an intracavernosal injection (ICI) of erectogenic medication. In patients with normal arterial inflow, peak systolic velocity (PSV) after ICI should exceed 30 cm/s. End diastolic flow (EDF) should be less than 5 cm/s. Arterial diameter should increase by 50% after injection. The erection obtained during PDUS should estimate the intactness of the veno-occlusive mechanism within the penis, but cannot exclude subtle forms of venous insufficiency, inhibited autonomic outflow, or totally psychogenic factors.

Treatment

Men with ED can have various etiologies. Diagnosis is key for appropriate counseling and treatment of the patient. Current non-experimental treatment modalities include lifestyle changes to minimize the risk of developing ED and oral medications, ICI, vacuum devices or intraurethral suppositories, and penile implant surgery. Finally, referral to a mental health professional can be considered to reduce anxiety around sexual performance.

Lifestyle Changes

Modification of various habits can result in decreased risk of developing ED. Various medical conditions, including obesity, diabetes, and cardiovascular disease, as well as lifestyle habits, including chronic alcohol use and tobacco use, can increase the risk of developing ED. In fact, the association of ED with cardiovascular disease is so strong that a diagnosis of ED in a young man with no comorbidities should be considered an indication for referral to a cardiologist for evaluation. Lifestyle modifications that have been linked with a decreased risk of developing ED include: an increase in physical activity along with weight loss and healthy diet, decreasing alcohol and tobacco use, improved glycemic control in diabetics, and minimizing stress, anxiety, and depression [3].

Medications

Phosphodiesterase 5 Inhibitors

Oral PDE5i medications act to inhibit the actions of PDE type 5, the enzyme responsible for cGMP breakdown in the corpora cavernosa. Inhibition of PDE5 results in continued smooth muscle relaxation and engorgement of the cavernosa with blood. There are various PDE5i medications, all with different speed of onset and varying half-lives, allowing the use of different PDE5i for men with differing sexual habits. For example, low-dose (5 mg tablet) tadalafil can be taken on a daily basis, allowing men to be more spontaneous in sexual encounters. On the other hand, sildenafil can be taken 1 h prior to sexual activity and allow for an 8-h window of opportunity for sex. FDA-approved PDE5i medications include sildenafil, vardenafil, tadalafil, and avanafil.

PDE5i are generally well-tolerated; however, relatively common sides effects include headache, flushing, dyspepsia, nasal congestion or rhinitis, and myalgias (mostly with tadalafil). Priapism, or erection lasting longer than 4 h, is an extremely rare side effect of PDE5i. There are several absolute and relative contraindications to taking PDE5i. Absolute contraindications include the use of nitrate-containing medications, as the concomitant administration of PDE5i and nitrates can result in life-threatening hypotension. Men with significant cardiovascular comorbidities, including recent serious cardiovascular events, uncontrolled hypertension, or unstable angina, should first be evaluated by a cardiologist prior to initiation of PDE5i for treatment of ED.

Vacuum Erection Device

Vacuum erection or constriction devices (VED/VCD) are non-medicinal, non-surgical means to obtain an erection. A plastic cylinder is placed over a lubricated, flaccid penis until the end of the cylinder is tightly fit against the abdominal wall. A vacuum is generated within the cylinder using a pump, which engorges the penis with venous blood, and an erection is produced [3]. To maintain the erection, a constriction band is placed at the base of the penis, and can be left in place for a maximum of 30 min. Although reports have described VED/VCD to be an effective means to achieve erection, numerous unfavorable side effects often deter men with ED from using the device. These side effects include coldness or numbness around the penis, bruising, pivoting at the base of the penis, pain or discomfort from the device or constriction band, and decreased ability to orgasm [3]. The use of VED also requires good manual dexterity to use the device appropriately.

Intraurethral Alprostadil

Alprostadil (prostaglandin E1) can be administered as an intraurethral suppository and has been found to be effective in approximately 40% of men with ED of various etiologies [3]. The medication is absorbed by the cavernosal tissue through vascular communication within the corpus spongiosum. Patients are taught how to insert the suppository in the clinic so that any occurrence of adverse side effects, such as urethral bleeding, vasovagal reflex, hypotension, and priapism (rare), can be monitored. Dosing typically starts at 500 μg and can be titrated to 1000 μf depending on the patient's response. The most common side effect of medication administration is urethral discomfort or pain.

Intracavernosal Injections

ICI can be performed if patients fail other ED medications. The benefit of ICI is that there is generally a high rate of efficacy and a low rate of side effects. Importantly, nerve function is not required for ICI to be efficacious; however, the cavernosal smooth muscle must be healthy and intact for ICI to work. Currently, different formulations of ICI available for use include prostaglandin E1 monotherapy (alprostadil), bimix (a combination of papaverine and phentolamine), trimix (a combination of papaverine, phentolamine, and prostaglandin E1), and quadmix (a combination of papaverine, phentolamine, prostaglandin E1, and atropine). There is an approximate 60% efficacy rate with prostaglandin E1 therapy alone, compared with an approximate 80% efficacy rate with trimix therapy in the same treatment group [4]. Failure of ICI is typically associated with collagenization of the cavernosal smooth muscle, which then results in venous leak and failure of medications including ICI. The main adverse effect of ICI is the development of ischemic priapism, or an erection lasting longer than 4 h that is not related to sexual stimulation and can occur even after orgasm or ejaculation. Ischemic priapism is a dangerous condition that requires urgent action because although an erection is occurring, no oxygenated blood is reaching the cavernosal smooth muscle, which can lead to irreversible changes and ultimately erectile dysfunction. Men being initiated on ICI should be taught proper injection technique prior to use.

Surgery

Penile implant surgery is typically reserved for patients who fail to achieve adequate erectile function with first- or second-line treatment options for ED. However, after appropriate counseling, patients who do not wish to pursue second-line options can opt for a penile implant. There are various types of penile implants, with 3-piece inflatable implants being the most commonly placed. With appropriate preoperative counseling of postoperative expectations, satisfaction rates after penile implant surgery are high (typically greater than 95%) due to the fact that these devices are

reliable and allow for spontaneous sexual activity. After appropriate placement of an implant, the penile shaft can become 100% rigid by pumping up the device, which typically takes 30–60 s [5]. The main disadvantages to penile implant surgery include the need for general anesthesia and the risks of surgery. The risk of infection is typically less than 2%, but higher in cases of revision and replacement or if the patient is diabetic, and mechanical failure of the implant is typically 15% within the first 10 years after placement. It is important to provide patients with adequate resources including instructional videos, device demonstration, and access to patient advocates, and involve their partners in surgical counseling prior to making the decision to have penile implant surgery.

In conclusion, erectile dysfunction can be due to various etiologies; however, there are many viable treatment options available. Importantly, ED can be a harbinger of underlying cardiovascular disease, and these men must be referred to cardiology for evaluation. It also is important to stress that a fraction of patients will present with erectile dysfunction that cannot be explained by any of the described etiologies—and in these men, it is important to note that psychological stressors (anxiety and depression) may play a big role in their pathology. In these men, it is imperative to treat their symptoms (i.e., prescribing PDE5i or other medication) and to refer them to a mental health professional who can assist with their psychological stressors. Finally, it is important to note that although there are various treatment options, the final treatment plan offered should be based on the desires of the patient and their partner, with the risks, benefits, and alternatives thoroughly discussed.

References

1. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. J Urol. 1994;151(1):54–61.
2. Burnett AL, Nehra A, Breau RH, Culkin DJ, Faraday MM, Hakim LS, et al. Erectile dysfunction: AUA guideline. J Urol. 2018;200(3):633–41.
3. Raina R, Pahlajani G, Agarwal A, Zippe CD. Treatment of erectile dysfunction: update. Am J Mens Health. 2007;1(2):126–38.
4. Mulhall JP, Stahl PJ, Stember DS. Clinical care pathways in andrology. New York, NY: Springer Science; 2014.
5. Habous M, Tal R, Tealab A, Azi M, Sherif H, Mahmoud S, et al. Predictors of satisfaction in men after penile implant surgery. J Sex Med. 2018;15(8):1180–6.

Chapter 25
Varicocele

Check for updates

Nahid Punjani and Marc Goldstein

Case

A 28-year-old male (DC) presents to a urologist with scrotal discomfort. Upon presentation he also describes that he and his wife have had difficulty conceiving. He has no prior semen analysis. He has also noticed some decreased energy and muscle mass. He and his 26-year-old wife, a nulligravida, would like two to three children. They discontinued contraception 18 months ago.

DC has had scrotal pain for 1 year. He first noticed a bulge in the scrotum 3 years ago, but that was not bothersome. The pain is described as a constant dull ache. The discomfort is improved with supportive underwear. He does not take any medication for the pain. He has no other associated symptoms such as voiding issues. A doctor told him he had a left varicocele when he was a teenager, but that it was nothing to worry about.

DC is healthy otherwise and does not have any known drug allergy or take any medication. There is no history of urinary tract or sexually transmitted infections. Other than a neonatal circumcision, he has no history of surgery. He is a non-smoker, consumes alcohol socially and denies any recreational drug use. He is a lawyer and recently married. He is the youngest of four healthy siblings and has no known family history of infertility.

With respect to fertility, they have been attempting to conceive for 1.5 years with unprotected intercourse. His wife is healthy and was previously seen by her obstetrician-gynecologist for suspected endometriosis, but her work-up was completely negative. Menstrual periods have been regular throughout life. DC has

N. Punjani
Department of Urology, Weill Medical College of Cornell University, New York, NY, USA

M. Goldstein (✉)
Male Reproductive Medicine and Surgery, Weill Medical College of Cornell University, New York, NY, USA
e-mail: mgoldst@med.cornell.edu

recently noticed decreased libido and disinterest in intercourse although they still manage to have unprotected intercourse around the time of ovulation. While he is usually able to achieve erections, he is not always able to maintain them, but when he does, he is able to ejaculate inside his partner. They deny use of any lubricants. DC also complains of fatigue and is less interested in many of his usual activities, especially finding it harder to keep up at the gym.

A first semen analysis revealed a concentration of seven million/mL, and total sperm count of 20 million. Sperm total motility was 30% and progressive motility 25%. Morphology was normal. Repeat semen analysis also revealed decrease concentration and motility. Volume and pH were within normal limit. His DNA fragmentation based on TUNEL was 20% (normal <7%).

General physical exam revealed no dysmorphic or syndromic features. His height was 6 ft. 3 in., and weight was 200 lbs. Abdominal examination was benign. There was no scar or mass felt. His genitourinary examination demonstrated a normal sized circumcised phallus. His meatus was in the orthotopic position and patent. Both testicles were normal sized with normal consistency. Epididymides were flat bilaterally and both vas deferens were identified. No hernia was detected. A grade 3b varicocele was appreciated on the left scrotum.

Bloodwork revealed a testosterone of 300 ng/dL. His scrotal ultrasound also confirmed a grade 3 varicocele on the left-hand side.

Treatment options were discussed and DC decided to proceed with a microsurgical left subinguinal varicocelectomy which was performed uneventfully. He had an uncomplicated recovery. His pain was improved and so was his libido post-surgery. Testosterone went up significantly to 410 ng/dL. Repeat semen analysis showed an improved sperm concentration of 35 million/mL and motility of 45%. DNA fragmentation index improved to 12%.

DC and his wife were able to achieve a pregnancy naturally within 6 months from surgery and gave birth to a healthy baby boy.

Discussion

In patients presenting with scrotal pain and infertility, the differential diagnoses may be broad. For scrotal pain, the differential diagnosis includes [1]:

- Testicular malignancy
- Orchitis
- Epididymitis
- Prostatitis
- Sexual transmitted disease
- Trauma
- Varicocele
- Hydrocele
- Intermittent testicular torsion

- Inguinal hernia
- Para-testicular mass
- Chronic orchialgia.

Whereas for suspected infertility the etiology may be multifactorial. Generally further information is required to narrow the differentials, but general categories include [2]:

- Pre-testicular

  – Hypogonadotropic hypogonadism (Kallman's, Prader-Willi, idiopathic)
  – Pituitary failure (malignancy, infectious, radiation, surgery)
  – Estrogen excess
  – Cortisol excess/deficiency (adrenal tumor, adrenal hyperplasia)

- Testicular

  – Congenital/Genetic/Chromosomal (Kleinfelter's, Down's, undescended testicles)
  – Acquired (varicocele, orchitis, radiation, trauma)

- Post-testicular

  – Obstruction (vasal obstruction, epididymal, ejaculatory duct obstruction)
  – Ejaculatory dysfunction (retrograde ejaculation, failure of emission)

A general and thorough evaluation is necessary for these patients. An appropriate history and physical examination will provide further insights into the etiology of the pain. A semen analysis may be indicated when there is difficulty with conception. A focused infertility and sexual history involving the partner would also be critical. Furthermore, when additional symptoms such as those associated with hypogonadism (low testosterone) are present, this should be further explored. Patients should also receive an appropriate hormonal profile and other imaging investigations as required.

History

For an individual such as DC with scrotal pain, it is important to discern an appropriate pain history. Given suspected infertility, history must include the duration of infertility and whether it is primary or secondary (having previously fathered a child or having been responsible for a pregnancy).

- Pain History:

  – Onset: sudden or gradual, intermittent or constant
  – Duration of pain
  – Quality of the pain: dull or sharp
  – Location of pain

- Exacerbating factors: urination, erection, ejaculation
- Relieving factors
- Associated symptoms: fever, chills, urinary frequency, dysuria, urethral discharge, scrotal swelling, change in height or position of the testis
- Methods used for relief

• Infertility History [3]:

- How many children would they like to have
- Primary or secondary infertility
- Duration of infertility (standard definition of >12 months in young couples <35 years old)
- Partner history (age, gynecological history, previous pregnancies, work-up by gynecologist or reproductive endocrinologist- AMH, ultrasound, HSG)
- History of childhood illness (i.e. orchitis)
- History of undescended testicle
- History of inguinal or pelvic surgery
- History of infertility surgery (i.e. vasectomy or varicocelectomy)
- History of pelvic or genital trauma
- History of infections of the genitourinary tract (including sexually transmitted infections)
- Exposure to gonadotoxins (i.e., medications, environmental exposures, chemotherapy, radiation)
- Steroid or testosterone use
- Signs and symptoms of any genetic conditions
- History of abnormal vision or visual changes
- Family history of fertility issues

• Sexual History [3]:

- Sexual drive/desire
- History of erectile dysfunction
- History of ejaculatory dysfunction
- Intercourse frequency and intravaginal ejaculation
- Use and type of lubricants
- Use of contraception.

• Hypogonadism (low testosterone) History:

- Decreased libido
- Decreased mood
- Loss of muscle mass
- Fatigue
- Decreased energy
- Hot sweats
- Altered cognition
- Change in body hair distribution

Semen Analysis

Patients with suspected infertility should have an appropriately collected semen analysis and then have it repeated. The sample should be obtained with self-stimulation, abstinence period of 2–5 days, and no lubrication. Two samples should be obtained at least 4 weeks apart. Samples should be examined with microscopic examination by a skilled technician (Table 25.1). Critical components of the semen analysis include:

Physical Examination

A physical examination for patients with pain and infertility is very helpful for diagnosis. This should include a general examination, as well as focused exam on the genitourinary organs [2]:

- General appearance (congenital abnormalities, secondary sex characteristics)
- Body habitus and gynecomastia
- Abdomen and inguinal areas for scars
- Phallic examination (circumcision status, meatal opening)
- Examination of the vas deferens (unilateral or bilateral)
- Testicular examination (size and consistency)
- Epididymal examination (dilated or flat)
- Cord examination (varicoceles, including grade or hernias)
- Digital rectal examination

Table 25.1 WHO 2010 semen parameters [4]

Semen parameter	World Health Organization 2010 values	Abnormality
Volume (mL)	1.5–6	
Total sperm count (TSC) (million)	39	Azoospermia (TSC = 0) Severe Oligospermia
Concentration (million/mL)	15	(TSC<5 millions) Oligospermia (TSC<15 millions)
Progressive motility (%)	32	Asthenospermia
Total motility (%)	40	
Normal morphology (%) (strict)	4–14%	Teratozoospermia
Vitality (%)	58	
Leukocyte count (million/mL)	<1.0	

Proper Varicocele Examination

- Patient should be in a quiet room and heating pad placed on the scrotum (both a cold room and anxiety may make examination challenging)
- Examination needs to be completed both supine and standing
- Testicular size should be estimated with an orchidometer
- After visualization for grossly dilated veins, the scrotum should be squeezed between fingers above the testicle in the area of the cord. This should be repeated with Valsalva
- Any veins should be palpated and felt as patient changes from standing to supine; abnormal veins will collapse and normal veins will stay palpable
- The exam should be repeated while supine
- Grading (modified from Dubin and Amelar)

  - Grade 1—palpable impulse with Valsalva maneuver
  - Grade 2—palpable impulse with Valsalva maneuver and palpable tortuosity of cord veins
  - Grade 3—visible impulse on Valsalva and when upright without Valsalva

    Grade 3a—just visible
    Grade 3b—fills most of the hemiscrotum
    Grade 3c—fills entire scrotum (Fig 25.1).

Fig. 25.1 Diagram illustrating a grade 3 varicocele

Laboratory Investigations

In a patient with signs and symptoms of hypogonadism as well as infertility, a baseline testosterone is warranted. Serum measurement (normal range 270–1070 ng/dL), preferably obtained in the morning due to the natural diurnal variation, provides an assessment of testicular function and/or failure.

The addition of follicle-stimulating hormone (FSH) is useful in men with sperm concentration <10 million/mL, altered sexual function or clinical signs of an endocrinopathy according to the AUA guidelines [3]. An elevated serum FSH (normal <8 IU/mL) is indicative of impaired sperm production as opposed to obstruction. Other hormones may be ordered based on clinical indication or at the discretion of the provider including luteinizing hormone (LH), thyroid stimulating hormone (TSH), estradiol and prolactin.

Additional Investigations for the Pain Include

- Scrotal ultrasound: for varicocele assessment, presence of scrotal mass, hydrocele or spermatocele. Numerous grading schemes exist, but generally veins >3 mm are considered clinically significant [5]. The Sarteschi classification includes the following [6]:

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

- Semen culture after antibacterial skin prep for gonorrhea, chlamydia, and ureaplasma if there is a suspected infectious etiology contributing to the pain. Urine culture and sensitivities should be ordered only if urinary symptoms are present.
- Abdominal imaging: For patients with an isolated right sided varicocele, especially ones that do not collapse when supine, further imaging of the abdomen to rule out a renal tumor or other retroperitoneal process.

Other Investigations for Infertility in the Context of Varicoceles

- DNA Fragmentation [7]: Patients with varicocele often have abnormal levels of DNA fragmentation. Studies have shown that repair of varicocele may improve DNA fragmentation levels. Standard assays for assessment include the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and Sperm Chromatin Structure Assay (SCSA). These two assays correlate 70% of the time [8].
- Anti-sperm antibody (ASA): Some studies have shown a small subset of patients with varicocele have anti-bodies to sperm, however, the clinical significance and correlation has yet to be clearly determined. High levels of ASA is diagnostic of obstruction [9].

Treatment

Indications for Varicocele Treatment Include the Following

- Symptoms including recurrent and persistent pain—reported rates of pain resolution vary from 50 to 90% following varicocele repair.
- Infertility—numerous studies have shown that varicocele treatment improves semen parameters including concentration and motility in a majority of men, and improved pregnancy outcomes with or without ART after repair [10].
- Patient preference (i.e. cosmetic reasons)—large varicoceles may appear as a "bag of worms" in the scrotum, and grossly dilated veins may be visible and bothersome to patients.
- Testicular size discrepancy (pediatric population)—variable definitions have been used for a significant discrepancy, some up to 20% difference. In adolescents, treatment usually results in a "catch-up" growth.
- Low testosterone—studies have shown that men with low testosterone have improvements of up to 14% average after their varicocele is repaired [11].

Numerous Treatments for Varicocele Exist

- Embolization by interventional radiology—this requires entry at the jugular or femoral vessels whereby veins are identified and occluded with tiny coils. Embolization has an ~80% initial success rate, but is associated with a high risk of recanalization, resulting in late recurrence.

- Microsurgical varicocelectomy—this is completed either through an inguinal or preferably subinguinal incision. The cord is delivered and care is taken to preserve arterial supply in the cord with the utilization of a doppler ultrasound probe. All visible veins are taken while lymphatics and associated nerves are spared. Following completion of vein ligation in the cord, external spermatic veins and any venous drainage from the gubernaculum should be taken. The veins of the vas deferens provide venous return after successful repair. This is the technique currently recommended in the AUA guidelines for treatment of varicocele.
- Non-microsurgical varicocelectomy—this is completed in the same fashion as the microsurgical method with or without surgical loupes. This technique may be approached inguinally or subinguinally.
- Retroperitoneal varicocelectomy—an open procedure performed using the Palomo technique, which includes a Gibson incision with ligation of the internal spermatic vein between the anterior superior iliac spine and renal vein.
- Laparoscopic varicocelectomy—this is completed transperitoneally either with the use of three ports or a single port surgical method. The peritoneum is opened proximal to the internal inguinal ring and spermatic vessels are dissected out with the veins ligated. The artery may or may not be spared.
- Retroperitoneoscopic varicocelectomy—this is completed through an incision below the 12th rib to create access to the retroperitoneal space. Spermatic vessels are dissected off the peritoneum and veins are ligated.
- Robotic assisted varicocelectomy—this technique is performed with the same steps as a subinguinal varicocelectomy but utilizing robotic arms to assist and maneuver through the cord and for vessel identification and ligation (Fig. 25.2 and Table 25.2).

In conclusion, as in our case, prompt and careful attention to a complaint of scrotal pain and infertility resulted in an accurate diagnosis of varicocele. Semen analysis, DFI, hormone evaluation, and imaging studies delineated the severity of the varicocele, allowing varicocelectomy to be performed which alleviated pain and achieved fertility at the end.
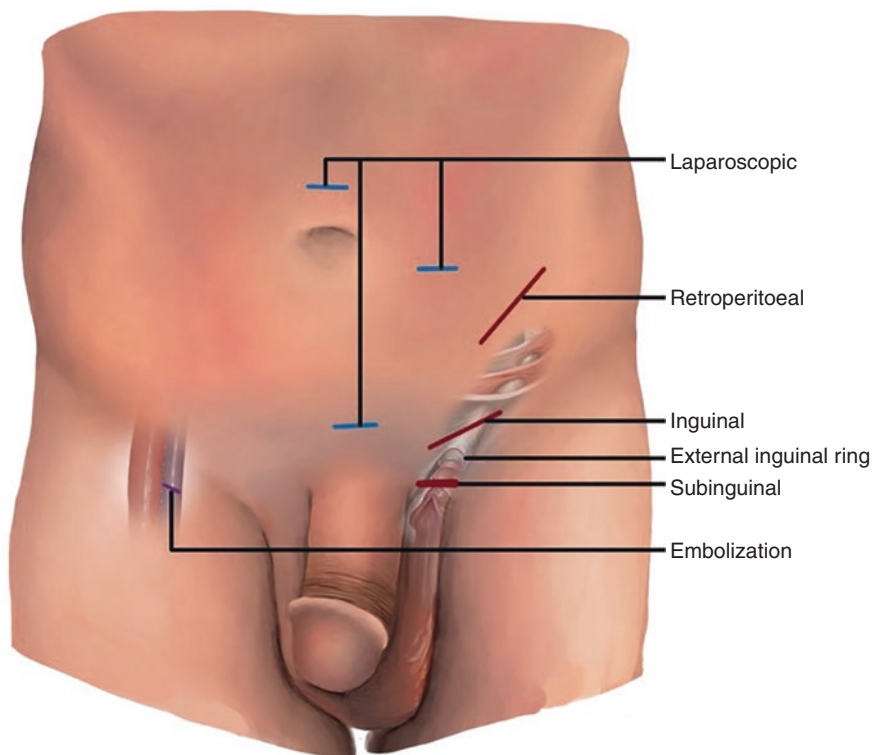
Fig. 25.2 Approximate locations of incisions for the various technical approaches for varicocele correction

Table 25.2 Comparison of outcomes of various treatment options for varicocele [12]

Technique	Recurrence/ persistence	Hydrocele formation	Spontaneous pregnancy
Embolization	3–11%	–	33.2%
Open inguinal (non-microsurgical)	2.6%	7%	36%
Microsurgical subinguinal	0–2%	0.4%	42%
Retroperitoneal	9–45%	8%	38%
Laparoscopic	3–15%	2.8%	30%

References

1. Sigalos JT, Pastuszak AW. Chronic orchialgia: epidemiology, diagnosis and evaluation. Transl Androl Urol. 2017;6(Suppl 1):S37–43.
2. Jarvi K, Lo K, Grober E, Fischer MV, Grantmyre J, Zini A, et al. The workup and management of azoospermic males. Can Urol Assoc J. 2015;9(7–8):229–35.
3. Jarow J, Sigman M, Kolettis PN, Lipshultz LR, Nangia AK, Naughton CK, et al. The optimal evaluation of the infertile male: American Urologic Association—best practice statements. Linthicum, MD: American Urological Association; 2010.
4. Cooper TG, Noonan E, von Eckardstein S, Auger J, Gordon Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16(3):231–45.
5. Practice Committee of the American Society for reproductive medicine and Society for Male Reproduction and Urology. Report on varicocele and infertility: a committee opinion. Fertil Steril. 2014;102(6):1556–60.
6. Belay RE, Huang GO, Shen JK, Ko EY. Diagnosis of clinical and subclinical varicocele: how has it evolved? Asian J Androl. 2016;18(2):182–5.
7. Telli O, Sarici H, Kabar M, Ozgur BC, Resorlu B, Bozkurt S. Does varicocelectomy affect DNA fragmentation in infertile patients? Indian J Urol. 2015;31(2):116–9.
8. Stahl PJ, Cogan C, Mehta A, Bolyakov A, Paduch D, Goldstein M. Concordance among sperm deoxyribonucleic acid integrity assays and semen parameters. Fertil Steril. 2015;104(1):56–61.
9. Lee R, Goldstein M, Ullery BW, Ehrlich J, Soares M, Razzano RA, et al. Value of serum anti-sperm antibodies in diagnosing obstructive azoospermia. J Urol. 2009;181(1):264–9.
10. Esteves SC, Roque M, Agarwal A. Outcome of assisted reproductive technology in men with treated and untreated varicocele: systematic review and meta-analysis. Asian J Androl. 2016;18(2):254–8.
11. Tanrikut C, Goldstein M, Rosoff JS, Lee RK, Nelson CJ, Mulhall JP. Varicocele as a risk factor for androgen deficiency and effect of repair. BJU Int. 2011;108(9):1480–4.
12. Cayan S, Shavakhabov S, Kadioglu A. Treatment of palpable varicocele in infertile men: a meta-analysis to define the best technique. J Androl. 2009;30(1):33–40.

Chapter 26
Azoospermia

Nahid Punjani and Peter Schlegel

Case

A 30-year-old male patient (BX) with history of infertility for over 1 year presents after having completed a semen analysis which displayed a volume of 3 mL with normal viscosity and appearance, pH 7.5 but concentration was zero. No sperm was seen even after centrifugation. He presents with his wife, a 28-year-old nulligravida.

He has no known allergies and is not on any medication. He is otherwise healthy with no previous surgeries. He is an only child who has never smoked cigarettes or done any recreational drug but he consumed alcohol occasionally. He works as a computer software developer. He is married and never fathered any children. There is no family history of fertility issues or significant medical conditions.

Given his normal semen volume and pH, with azoospermia, the differential initially includes a testicular or pre-testicular etiology, while a post-testicular cause such as obstruction is less likely.

His partner is healthy and reports regular monthly menses. She denies any gynecologic issues and has had a completely normal evaluation by her gynecologist which includes AMH, TSH, and prolactin levels as well as a hysterosalpingogram (HSG).

BX has no history of any infection as a child or any childhood surgical procedures. He had been informed that his testicles were properly descended. He has no history of previous inguinal, pelvic or urologic surgery. He has never had a sexually transmitted disease. Neither does he have any hazardous occupational, toxin nor radiation exposure.

BX has a strong sexual drive and desire. He is able to achieve and maintain erections for penetrative intercourse and ejaculate inside his partner. They have

N. Punjani · P. Schlegel (✉)
Department of Urology, Weill Medical College of Cornell University, New York, NY, USA
e-mail: pnschleg@med.cornell.edu

unprotected intercourse every other day around the time of ovulation. They do not use any lubricant.

On general examination, BX had no dysmorphic or syndromic features. His height was 5 ft. 10 in., and weight was 170 lbs. He has a normal muscular build with no evidence of gynecomastia. Abdominal examination did not reveal any scar, mass or evidence of hernia repair. His genitourinary examination revealed a normal-sized, circumcised phallus. His meatus was in the orthotopic position and patent. Testicular examination demonstrated a volume of 12 cc bilaterally that were normal in consistency. Epididymis was flat and both vas deferens were palpable.

Bloodwork revealed a normal testosterone of 600 ng/dL but FSH level was 21 IU/L. Given his high FSH level and the clinical presentation, which was suggestive of non-obstructive azoospermia (NOA), a karyotype and Y-microdeletion studies were performed. His karyotype was 46, XY and Y-chromosomal studies revealed an AZFc deletion.

BX underwent genetic counseling regarding his AZFc deletion. This couple remained interested in having children and underwent in vitro fertilization (IVF) and micro-TESE (microsurgical testicular sperm extraction) on the day of oocyte retrieval. Sperm retrieved were used for intracytoplasmic sperm injection (ICSI). A viable pregnancy was achieved after a single embryo transfer which led to a term delivery of a healthy child.

Discussion

Azoospermia may be etiologically grouped into three different categories, pre-testicular (2%), testicular (49–93%), and post-testicular (7–51%) [1]. It can also be categorized as obstructive (OA) or non-obstructive (NOA). Pre-testicular etiologies are the least common and often relate to underlying endocrine abnormalities including issues with their hypothalamic-pituitary axis which impact upon spermatogenesis. They are generally classified as secondary testicular failure. A list of some specific conditions includes [1, 2]:

- Congenital conditions (i.e., Kallmann syndrome, non-Kallmann idiopathic hypogonadotropic hypogonadism, Moebius syndrome, Prader–Willi syndrome)
- Hyperprolactinemia
- Exogenous Testosterone (replacement therapy and anabolic steroids)
- Hypothalamic and pituitary disorders (i.e., secondary to radiation, medications)
- Estrogen excess
- Adrenal tumor

Testicular etiologies are most common. These patients have spermatogenic abnormalities within the testes themselves. They are generally classified as primary testicular failure. Many of these are either congenital or acquired [1, 2]:

- Congenital

    - Undescended testicle
    - Noonan syndrome
    - Kleinfelter syndrome
    - XX male
    - XYY male

- Acquired

    - Trauma
    - Varicocele
    - Infection (i.e., orchitis)
    - Gonadotoxin exposure, including chemotherapy
    - Radiation

Post-testicular etiologies are relatively common and are often attributed to obstructive etiologies or ejaculatory dysfunction; some examples include [1, 2] the following:

- Obstruction

    - Congenital bilateral absence of the vas deferens
    - Vasal obstruction
    - Epididymal obstruction
    - Ejaculatory duct obstruction

- Ejaculatory Dysfunction

    - Retrograde ejaculation (i.e., secondary to medication or diabetes)
    - Failure of emission (i.e., neurologic injury)

In general, all azoospermic men should undergo a thorough infertility and sexual history in addition to their general medical and surgical history. All patients must also receive a focused physical exam as well as appropriate testing including repeat semen analysis, hormonal profiles, and other investigations as needed.

Semen Analysis

According to the American Urologic Association (AUA) Guidelines it is recommended that azoospermia can only be diagnosed after observing no sperm on at least two centrifuged semen sample [3]. Centrifugation should be performed at maximal speed (1500–1800 g for 15 min at room temperature) with subsequent microscopic examination by a skilled technician [4]. The sample should be obtained by masturbation without any lubricant and with an abstinence period of 2–7 days. Sample collections should be at least 4 weeks apart. Please refer to Chap. 25 regarding WHO 2010 Semen Parameters [5].

History and Physical Examination

A pertinent history and physical examination will help make the correct diagnosis and ascertain other possible etiologies of infertility [1, 3]:

- Infertility History:

  - Primary or secondary infertility
  - Duration of infertility (standard definition of >12 months)
  - Partner history (i.e., age, gynecological history, work-up by reproductive endocrinologist, previous pregnancies with a different partner)
  - History of childhood illness (i.e., orchitis)
  - History of undescended testicle(s)
  - History of inguinal or pelvic surgery
  - History of genitourinary surgery (i.e., vasectomy)
  - History of pelvic or genital trauma
  - History of infections of the genitourinary tract (including sexually transmitted infections)
  - Exposure to gonadotoxins (i.e., medications, environmental exposures, chemotherapy, radiation)
  - Steroid or testosterone use
  - Signs and symptoms of any genetic conditions
  - Delayed puberty
  - History of abnormal vision or visual changes
  - Family history of fertility issues

- Sexual History:

  - History of erectile dysfunction
  - History of ejaculatory dysfunction
  - Sexual drive/desire
  - Coital frequency
  - Use and type of lubricants
  - Use of contraception

- Physical Examination

  - General appearance (congenital abnormalities, secondary sex characteristics)
  - Body habitus and gynecomastia
  - Abdominal and inguinal exam for scars
  - Phallic examination (circumcision status, meatal opening)
  - Presence or absence of the vas deferens (unilateral or bilateral)
  - Testicular examination (size and consistency)
  - Epididymal examination (dilated or flat)
  - Cord examination (varicoceles or hernias)
  - Digital rectal exam (midline prostatic cyst)

Absence of the vas deferens (unilateral or bilateral), dilated epididymis or clinical findings suggestive of ejaculatory duct obstruction may indicate obstructive azoospermia. Individuals with the absence of the vas deferens may have or are carriers of the cystic fibrosis gene and should have appropriate testing as discussed in the next section. The majority of patients with obstructive azoospermia will generally have testicular length >4.6 cm [4]. Patients with primary or secondary testicular failure may have small and soft testicles (<4.6 cm) suggestive of non-obstructive azoospermia [4].

Laboratory Investigations

Initial hormonal evaluation according to the AUA Guidelines includes serum levels of testosterone and follicle-stimulating hormone (FSH) [3]. This provides further information to determine if a patient has obstructive or non-obstructive azoospermia (Table 26.1). Other hormones that may be ordered include luteinizing hormone (LH), thyroid stimulating hormone (TSH), estradiol levels, and prolactin as clinically indicated [2].

- Testosterone: serum measurement, preferably obtained in the morning due to the natural diurnal variation, provides an assessment of testicular function and/or failure (normal range 300–800 ng/dL).
- FSH: serum measurement may inform hyper- (primary testicular failure) or hypogonadotropic (secondary testicular failure) hypogonadism (normal range <7.6 mIU/mL).

Up to 96% of patients with obstructive azoospermia have an FSH <7.6 mIU/mL and over 90% of those with non-obstructive azoospermia will have an FSH >7.6 mIU/mL [4].

Additionally, in obstructive azoospermia or retrograde ejaculation specific investigations include:

Table 26.1 Summary of various evaluation tools and etiologies of azoospermia

Parameter/testing	Pre-testicular	Testicular	Post-testicular
Testis volume/size	⇓	⇓/N	N
Semen volume	⇓/N	N	⇓/N
Semen pH	N	N	⇓/N
Testosterone	⇓	⇓/N	N
FSH	⇓	⇑/N	N
LH	⇓	⇑/N	N
CFTR	N	N	N/Abn
Karyotype	N/Abn	N/Abn	N
Y-microdeletion	N	N/Abn	N

- Post-ejaculatory urinalysis [1]: utilized for the assessment of men with low semen volume, including aspermia, for retrograde ejaculation. Patients are asked to void after ejaculation and urine is subsequently centrifuged and assessed for the presence of sperm. A tablet of sodium bicarbonate is taken beforehand to help alkalinize the urine as the acidic urine environment may be harmful to sperm. Some have historically suggested placement of appropriate media in the bladder before ejaculation in an effort to protect sperm [6].
- Ultrasound (transrectal and/or scrotal) [1]: for assessment of varicocele, ejaculatory duct dilation or any other suspected genitourinary abnormality.
- Cystic fibrosis transmembrane conductance regulator (CFTR) Testing [4]: serum measurement for patients with CAVD either unilaterally or bilaterally to assess for the presence of cystic fibrosis gene mutations. Partners should also be tested and genetic counseling referral should be initiated where appropriate. Testing for the 5T allele is also indicated for men with BCAVD.
- Abdominal ultrasound [4]: performed in patients with BCAVD and negative CFTR testing as patients may have associated renal anomalies (i.e., renal agenesis).

Genetic Work-Up and Implications

Karyotype and Y-microdeletion testing should be offered to all patients with non-obstructive azoospermia or severe oligospermia (<five million sperm/mL), based on AUA guidelines [4].

The most common chromosomal abnormality in men with infertility is Kleinfelter syndrome (47XXY or 46XY with mosaicism). The phenotype is variable from complete azoospermia to compromised sperm production [4].

Microdeletions may occur in the long arm of the Y chromosome, which are often due to ampliconic and palindromic sequences in this region. Three commonly detected regions of deletions may occur including AZFa, AZFb, and AZFc [7]. Complete microdeletions of AZFa or AZFb regions will reliably predict azoospermia with no reasonable chance of surgical sperm retrieval. AZFa specifically tends to result in uniform Sertoli cell only syndrome. However, patients with AZFc microdeletion have been reported to have sperm in the ejaculate in a majority of cases with surgical sperm retrieval rates of up to 50–70% [8].

Testicular Biopsy

Biopsy provides for a limited evaluation of testicular function and is only utilized to distinguish between obstructive and non-obstructive azoospermia, for example, in patients with normal testicular volume and normal or borderline elevated FSH [3]. This could be done as a therapeutic intervention as well, with sperm from removed

tissue utilized for assisted reproduction or cryopreserved. Various histologies may be found at the time of biopsy with subsequent sperm retrieval rates with TESE:

- Obstructive Azoospermia.

  - Normal spermatogenesis—very high sperm retrieval rates.

- Non-obstructive Azoospermia [4].

  - Hypospermatogenesis—sperm retrieval rates reached up to 79%
  - Maturation Arrest—sperm retrieval rates reached up to 47%.
  - Sertoli Cell Only—sperm retrieval rates reached up to 24%.

Treatment

Treatment options for patients with azoospermia are dictated by its etiology.
Obstructive azoospermia or retrograde ejaculation [1, 2]:

- Medical—utilization of medication to promote antegrade ejaculation in cases of retrograde ejaculation.
- Sperm retrieval for assisted reproductive technology—includes percutaneous or open sperm aspiration/retrieval.
- Minimally invasive procedure—for individuals who have failure to ejaculate due to neurological injury or spinal cord injury, techniques such as vibratory stimulation or electro-ejaculation may be utilized.
- Endoscopic surgery—transurethral resection of the ejaculatory ducts if there is evidence of ejaculatory duct obstruction.
- Surgical reconstruction—for obstructed patients, may require vasal or epididymal reconstruction to bypass the area of concern.
- Donor sperm, surrogacy, and/or adoption.

  Non-obstructive azoospermia [1, 2]:

- Medical—for those with hypothalamic-pituitary disorders.
- Sperm retrieval for assisted reproductive technology—includes percutaneous or open sperm aspiration/retrieval.
- Donor sperm, surrogacy and/or adoption.

  Medical options may include hormonal treatment in an attempt to increase spermatogenesis by enhancing endogenous testosterone production. Although controversial, there is some evidence suggesting the potential benefit of these treatments in non-obstructive azoospermic men [9]. Hormone values should be closely followed after initiation of these medications. Various medications have been implicated [10, 11] (Table 26.2).

- Pseudoephedrine: an alpha-agonist which attempts to convert retrograde ejaculation to antegrade ejaculation.

Table 26.2 Medications and dosages [11]

Medication	Dose
Pseudoephedrine	60 mg po qid
FSH	100–450 IU 2–3×/week
hCG	1500–5000 IU 2×/week
Anastrozole	1 mg/day
Letrozole	2.5 mg/day
Clomiphene citrate	12.5–50 mg/day
Tamoxifen	10–20 mg/day

- Recombinant FSH: works by stimulating FSH receptors in Sertoli cells to stimulate spermatogenesis in men with hypogonadotropic hypogonadism.
- Gonadotropins (i.e., human chorionic gonadotropin (hCG)): these medications directly stimulate LH receptors in Leydig cells and can therefore potentially improve. Spermatogenesis. This is commonly used in patients with low testosterone, especially those with hypogonadotropic hypogonadism, in whom there is a desire to raise intratesticular testosterone levels without compromising fertility.
- Aromatase Inhibitors (i.e., anastrozole, letrozole): these medications not only work through inhibition of the conversion of androgens to estrogens which increases testosterone levels, but also decrease estrogen feedback that increases GnRH and LH release. This is commonly used in patients with low serum testosterone-to-estradiol levels, with a threshold ratio of 10 as the lower limit of normal [12].
- Selective estrogen receptor modulator (i.e., clomiphene citrate, tamoxifen): these medications block the negative feedback of estrogen on the hypothalamic-pituitary axis thereby increasing FSH and LH production to stimulate testosterone and spermatogenesis. These are commonly used in patients with low testosterone levels.

Surgical Techniques for Sperm Retrieval [13]

- MESA (Microsurgical epididymal sperm aspiration): utilizes a scrotal incision where the testis is delivered and both testis and epididymis are observed under an operative microscope. The epididymis is examined for dilated tubules whereby sperm is aspirated. Multiple different segments of the epididymis are sampled, including the efferent ducts, where different sperm quality is typically found in each of the various segments of the epididymis. This is the gold standard for patients with obstruction.
- PESA (Percutaneous epididymal sperm aspiration): requires a needle to be placed transcutaneously into the caput of the epididymis whereby sperm is aspirated.
- TESA (Testicular sperm aspiration): utilization of a needle for transcutaneous aspiration of sperm from the testicle.

Table 26.3 Selected sperm
retrieval technique
success rates

Technique	Success rate
MESA	99–100% for OA [14, 15]
PESA	61–96% for OA [16, 17]
micro-TESE	45–63% for NOA [18]

- MicroTESE (Microsurgical testicular sperm extraction): this utilizes a midline scrotal incision to deliver the testicle which is then bivalued under the microscope and seminiferous tubules are examined for the presence of dilated tubules which may contain sperm. This will likely yield a greater chance of finding sperm compared to a random biopsy given the ability to directly identify dilated tubules. Processing of the specimen in the IVF laboratory is a critical component and may impact on the actual yields of sperm. This technique is often used for patients with non-obstructive azoospermia. (Note: up to 15% of men may have sperm on pre-operative semen analysis and therefore repeat semen analysis should be routinely performed before surgical retrieval) [4] (Table 26.3).

Sperm retrieved from these methods will be used in conjunction with assisted reproductive technology, either fresh at certain centers or may be cryopreserved for utilization at a later date.

Fresh Vs. Frozen Sperm

For patients undergoing sperm retrieval with obstructive azoospermia no differences have been observed in IVF success rates after use of fresh or cryopreserved sperm. However, some data highlights that in patients with non-obstructive azoospermia, overall results for fertilization and pregnancy rates are improved using fresh specimen synchronized with oocyte retrieval [4]. Using cryopreserved sperm in men with non-obstructive azoospermia has yielded poorer pregnancy outcomes often due to the limited number of sperm obtained or failure of sperm to survive freeze-thaw. Repeat sperm retrieval procedures in non-obstructive men are always not as successful.

Counseling

In the event when chromosomal abnormality, or genetic condition, is noted in azoospermic men, counseling should be provided. Patients with cystic fibrosis must also have genetic screening of the female partner due to the potential risk of disease in the offspring. For any Y-chromosome-related genetic aberrations, any male offspring would inherit the abnormality but they pose no risk of transmission to a female offspring. For Y-microdeletions, this would not apply to patients with AZFa and AZFb deletions s as these men would not be able to conceive.

In conclusion, azoospermia needs a careful and thorough approach to delineate if the etiology is obstructive versus non-obstructive. Obstructive causes offer better prognosis than non-obstructive ones. However, the condition in general presents as a clinical challenge in that one requires expertise in urology to procure sperm surgically, and an IVF program which is well equipped with a laboratory familiar with searching rare sperm potentially found in testicular specimens. Specific ICSI protocols are needed for injecting these fragile sperm into the oocytes.

In addition, female partners will have to be evaluated to rule out other factors accounting for infertility, e.g. advanced female age and uterine factors. In our case, the wife was very young and had a normal uterine cavity and therefore the couple enjoyed a much higher chance of success.

References

1. Jarvi K, Lo K, Grober E, Mak V, Fischer A, Grantmyre J, et al. The workup and management of azoospermic males. Can Urol Assoc J. 2015;9(7–8):229–35.
2. Cocuzza M, Alvarenga C, Pagani R. The epidemiology and etiology of azoospermia. Clinics (Sao Paulo). 2013;68(Suppl 1):15–26.
3. Jarow J, et al. The evaluation of the azoospermic male: American Urologic Association—best practice statements. Linthicum, MD: American Urologic Association; 2011.
4. Schlegel PN. Causes of azoospermia and their management. Reprod Fertil Dev. 2004;16(5):561–72.
5. Cooper TG, Noonan E, von Eckardstein S, Auger J, Gordon Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16(3):231–45.
6. Aust TR, Brookes S, Troup SA, Fraser WD, Lewis-Jones DI. Development and in vitro testing of a new method of urine preparation for retrograde ejaculation; the liverpool solution. Fertil Steril. 2008;89(4):885–91.
7. Kim SY, Kim HJ, Lee BY, Park SY, Lee HS, Seo JT. Y chromosome microdeletions in infertile men with non-obstructive azoospermia and severe oligozoospermia. J Reprod Infertil. 2017;18(3):307–15.
8. Stahl PJ, Masson P, Mielnik A, Marean MB, Schlegel PN, Paduch DA. A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia. Fertil Steril. 2010;94(5):1753–6.
9. Hussein A, Ozgok Y, Ross L, Rao P, Niederberger C. Optimization of spermatogenesis-regulating hormones in patients with non-obstructive azoospermia and its impact on sperm retrieval: a multicentre study. BJU Int. 2013;111:E110–4.
10. Shiraishi K. Hormonal therapy for non-obstructive azoospermia: basic and clinical perspectives. Reprod Med Biol. 2015;14(2):65–72.
11. Dabaja AA, Schlegel PN. Medical treatment of male infertility. Transl Androl Urol. 2014;3(1):9–16.
12. Schlegelm PN. Aromatase inhibitors for male infertility. Fertil Steril. 2012;98(6):1359–62.
13. Coward RM, Mills JN. A step-by-step guide to office-based sperm retrieval for obstructive azoospermia. Transl Androl Urol. 2017;6(4):730–44.
14. Schlegel PN, Palermo GD, Alikani M, Adler A, Reing AM, Cohen J, Rosenwaks Z. Micropuncture retrieval of epididymal sperm with in vitro fertilization: importance of in vitro micromanipulation techniques. Urology. 1995;46(2):238–41.

15. Bernie AM, Ramasamy R, Stember DS, Stahl PJ. Microsurgical epididymal sperm aspiration: indications, techniques and outcomes. Asian J Androl. 2013;15(1):40–3.
16. Meniru GI, Gorgy A, Batha J, Clarke RJ, Podsiadly BT, Craft IL. Studies of percutaneous epididymal sperm aspiration (PESA) and intracytoplasmic sperm injection. Hum Reprod Update. 1998;4(10:57–71.
17. Lin YM, Hsu CC, Kuo TC, Lin JS, Wang ST, Huang KE. Percutaneous epididymal sperm aspiration versus microsurgical epididymal sperm aspiration for irreparable obstructive azoospermia—experience with 100 cases. J Formos Med Assoc. 2000;99(6):459–65.
18. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. Hum Reprod. 1999;14(1):131–5.

Chapter 27
Advanced Sperm Function Testing

Stephanie Cheung, Alessandra Parrella, Philip Xie, Derek Keating, Owen Davis, Zev Rosenwaks, and Gianpiero D. Palermo

Case

We assessed a young couple who, despite having had unprotected intercourse for 2 years, remained unable to conceive.

The female partner, a 32-year-old teacher, is 5 ft 7 in. tall. When evaluated at our center, she weighed 234 pounds and had a body mass index of 36 kg/m^2. She denied drinking, smoking, and the use of recreational drugs. She is a carrier of Niemann–Pick disease. The patient's mother had a history of hypertension, and her father had adult-onset diabetes mellitus. She has two older sisters, and one of them has a child. She reports normal 28–32-day menstrual cycles and has never been pregnant. Evaluations included an anti-Müllerian hormone (AMH) level of 3.14 ng/mL, a normal pelvic sonogram and hysterosalpingogram.

The male partner, a 32-year-old convenience store owner, is Caucasian and had a BMI of 33.5 kg/m^2. His history indicated moderate alcohol consumption of five drinks per week and no recreational drug use. The patient admitted to smoking cigarettes daily for 13 years prior to quitting 2 months before this consultation. He denied any use of testosterone and had no history of medical diseases or surgical procedures. His father had a history of varicocele. He is not a carrier of Niemann–Pick disease. The male patient had no family history of diabetes or genetic diseases, although he has a brother with hearing loss. The patient was unaware of any fertility issues encountered by his brother or two sisters, all of whom are unmarried. He

S. Cheung · A. Parrella · P. Xie · D. Keating · O. Davis (✉) · Z. Rosenwaks · G. D. Palermo
Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY, USA
e-mail: stc3001@med.cornell.edu; phx3001@med.cornell.edu; okdavis@med.cornell.edu; zrosenw@med.cornell.edu; gdpalerm@med.cornell.edu

reported that an unmarried cousin had 0% normal sperm morphology but did not provide further details. The patient's prior semen analyses showed a concentration ranging from 9 to 35×10^6/mL, with 55–60% motility and consistently 0% normal sperm morphology which suggested globozoospermia. There was no evidence of Y microdeletions, and the patient's hormonal profile was normal.

The couple was diagnosed with severe male factor infertility and underwent 2 cycles of in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) at another center a few months prior to seeking treatment at our clinic. The first stimulation cycle was with 187.5 IU of urinary menotropin and 150 IU of follitropin daily, with a GnRH antagonist, leading to nine retrieved oocytes, of which six were mature. All six were injected with ICSI, and in spite of their incubation in calcium ionophore for 15 min after insemination, none fertilized. The second ICSI cycle with the same superovulation protocol occurred shortly thereafter. A total of 19 oocytes were retrieved this time, yielding seven metaphase II oocytes that underwent ICSI. Once again, despite incubation in calcium ionophore, no fertilization was reported.

This history of failed IVF cycles with ICSI, in spite of artificial oocyte activation (AOA), and the results of the previous semen analyses suggesting globozoospermia led us to suspect this diagnosis as well. After consultation with the reproductive endocrinologist, Andrology service and reproductive urologist, it was suggested to the couple that he should undergo a series of additional tests to assess sperm characteristics and function:

- *Aniline blue* to provide information on genome compaction during spermiogenesis
- *TUNEL* to assess the level of sperm DNA fragmentation
- *Sperm aneuploidy assay by FISH* to identify chromosomal abnormalities within the male gamete
- *PLCζ assay* to detect the presence of sperm cytosolic factor in the perinuclear theca of the sperm head to test the ability of the spermatozoa to activate the oocyte
- *Centrosome assessment* to identify the presence and extrapolate the eventual function of the centrosome
- *Transmission Electron Microscopy* to unequivocally confirm and identify the extent of globozoospermia, abnormal chromatin compaction, and absence of the acrosomal vesicle
- *Genetic and epigenetic profiling of the male gamete* to assess mutations and expression levels of genes related to globozoospermia and to gain insight into the oocyte activating potential of the spermatozoa

Semen Analysis: Semen analysis was performed on a fresh ejaculate produced within 4 days of abstinence. Volume, concentration, motility, progressive motility, and morphology were assessed according to the most recent WHO guidelines. Following liquefaction, the semen analysis revealed a volume of 4.3 mL, a concentration of 50×10^6/mL, 45% motility, and 41% progressive motility, all within the normal threshold. However, there was 0% normal sperm morphology. All spermatozoa assessed demonstrated spherical heads with absent acrosomes. More than

75% of the spermatozoa examined also displayed cytoplasmic residues in the mid-piece (Fig. 27.1).

Protamine Assay: During spermiogenesis, sperm chromatin undergoes compaction by the supercoiling of the DNA and the replacement of nuclear histones with the protamine core. This test provides valuable information on the integrity of the spermiogenic process within the seminiferous tubule. In this case, Aniline Blue assay was carried out on 200 spermatozoa and a threshold of <20%, as derived from a fertile control, was used. Since aniline blue stains nuclear histones but not protamines, immature sperm nuclei with residual histones will be stained dark blue. A total of 97 spermatozoa were deeply stained by aniline blue, indicating the presence of residual histones in 48.5% of the cells (Fig. 27.2). The test evidenced a remarkably compromised chromatin compaction.

Sperm Chromatin Fragmentation Assay: In mammalian spermatozoa, chromatin remodeling takes place during spermiogenesis involving the replacement of nucleosomal histones by protamines with an increase in histone acetylation, activity of the ubiquitin system, and a change in DNA topology resulting from the elimination of

Fig. 27.1 TestSimplet® slide of globozoospermic specimen
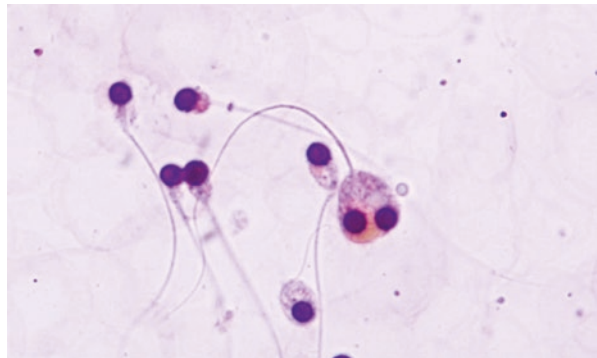
Fig. 27.2 Aniline Blue staining of globozoospermic specimen
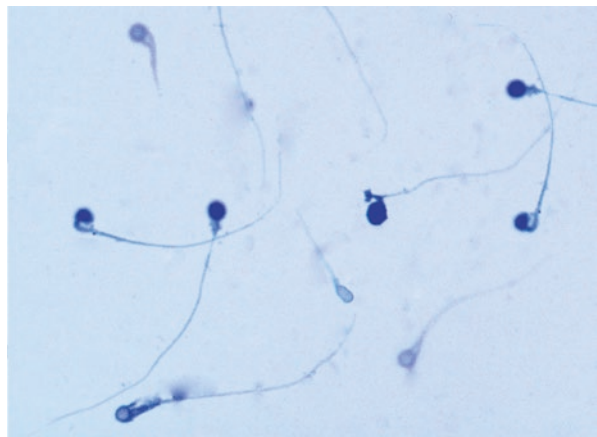
negative supercoiling through a process that seals and repairs the DNA phosphate backbone. At the spermatid stage, single and double stranded DNA breaks, which wrap around a protamine core to form the toroid structure, are repaired by specific enzymes such as topoisomerase II. This chromatin appears tightly condensed and transcriptionally inert; it is therefore highly resistant to digestion. However, histone-bound DNA persists in the linker region in between the toroid structures, representing at least 15–20% of the entire sperm genome. These areas, which are still very prone to DNA nicks and breaks due to reactive oxygen species present in the male genital tract, can be detected by sperm chromatin fragmentation (SCF) assays. SCF was assessed by labeling the 3′-OH ends using the TdT (terminal deoxynucleotidyl transferase)-mediated dUTP-biotin nick-end labeling (TUNEL) assay. A minimum of 500 spermatozoa were scored using fluorescent microscopy (Fig. 27.3), and an SCF of ≤15% was considered normal. In this patient, the TUNEL assay evidenced a mild increase in sperm DNA fragmentation of 16.8%.

Sperm Aneuploidy Assessment: In cases of unexplained infertility in which the male partner has seemingly normal sperm parameters, the genetics of the male gamete should be explored. In spite of the fact that sperm morphology does not directly relate to the genetics of the sperm cell, globozoospermic men have been thought to have a higher rate of sperm aneuploidy. Fluorescent in situ hybridization (FISH), a cytogenetic technique that utilizes fluorescent probes that bind to specific chromosomes, was applied to sperm cells to detect aneuploidy. This technique requires decondensation of the tightly compacted spermatozoal chromatin to access the DNA for probe hybridization. The multicolored labeled probe signals can then be visualized under fluorescent microscopy (Fig. 27.4). Nine chromosome (X, Y, 13, 15, 16, 17, 18, 21, 22) FISH was used to assess at least 1000 sperm cells for this case, with a normal threshold of 1.6%. The aneuploidy assessment by FISH for this patient revealed only a borderline increase of 1.9% total aneuploidy represented by gonosomal (XY, YY) and autosomal disomies for chromosomes 15, 16, and 17.

Fig. 27.3 TUNEL on globozoospermic specimen
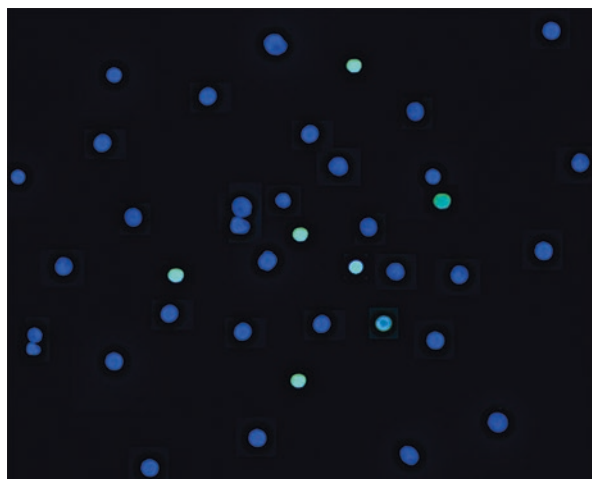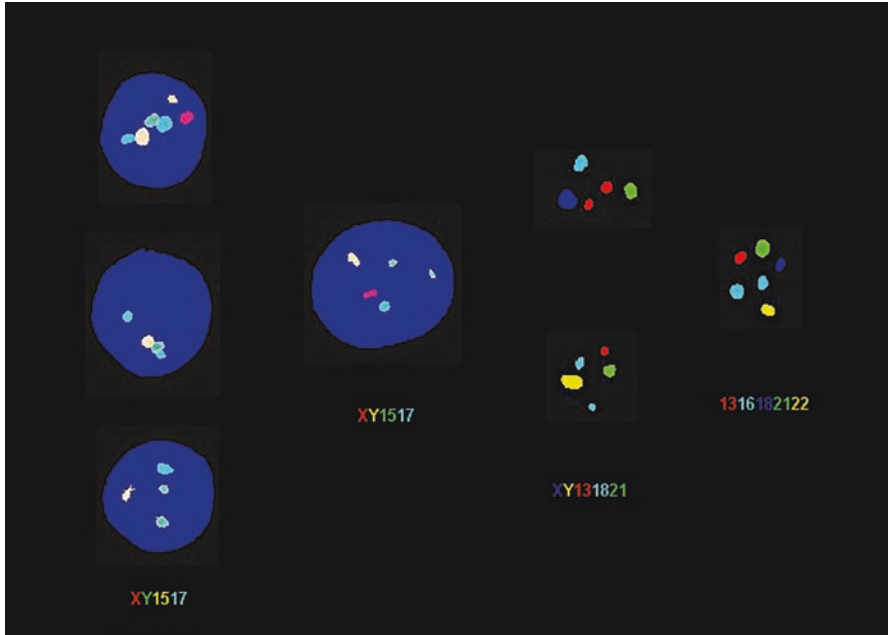
Fig. 27.4 FISH on globozoospermic specimen

Sperm Activating Factor Assessment: Upon fusion of the spermatozoon with the oolemma, the oocyte undergoes a biological process that leads to egg activations, which trigger meiotic progression from metaphase II arrest, cortical granule exocytosis, and transcription of the residual histone-bound region of the male genome to promote zygote development. Central to this process is phospholipase C-zeta (PLCζ), a sperm-specific protein, which when released into the oocyte upon sperm entry triggers the release of Ca^{2+} from the endoplasmic reticulum (ER) [1]. The delivery of PLCζ into the cytoplasm catalyzes the hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP2), forming the two messengers inositol 1,4,5 triphosphate (IP3) and diacylglycerol (DAG). The interaction of IP3 with its receptor on the ER opens the Ca^{2+} channels, releasing calcium in the cytoplasm of the oocytes [2]. For PLCζ assessment, spermatozoa were permeabilized by exposure to a detergent and incubated overnight with anti-human PLCζ antibodies. The percentage of sperm exhibiting PLCζ immunofluorescence was recorded for all sperm cells assessed ($n = 200$). The fertile control specimen displayed a strong fluorescence in the equatorial region of the head in more than 90% of the spermatozoa (Fig. 27.5). Our patient's specimen lacked the characteristic band on the sperm head in the majority of the cells, indicating an extremely reduced level of PLCζ, detectable in only 10.2% of the spermatozoa evaluated.

Centrosome Assessment: Embryonic cell division involves a cytoskeletal structure called the microtubule organizing center (MTOC). The MTOC stems from the centrosome, which consists of a pair of centrioles arranged perpendicularly and
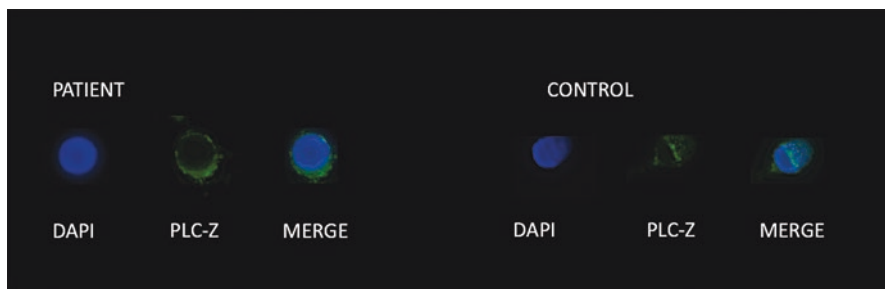
Fig. 27.5 PLCζ assessment on globozoospermic specimen

surrounded by fibrous pericentriolar material (PCM) that creates the aster and spindle fibers. Human spermatozoa have two centrioles: one is distal and supports the flagellum, and the other is proximal and is delivered into the egg [3]. The centrosome, by anchoring the sperm aster, is involved in the migration and juxtaposition of the male and female pronuclei. Indeed, the spermatozoon forms an aster around the midpiece, a radial structure composed of microtubules anchored to the proximal centriole. While the spermatozoon decondenses, the dimension of the aster increases, jutting out from the male genome and adjoining the female pronucleus toward the center of the cell. The centrosome is also responsible for the regulation of cell polarity of the first mitotic cell division. After syngamy, the centrioles duplicate into daughter centrioles that migrate to the opposite poles of the first mitotic spindle during metaphase. Once the anaphase and telophase stages are complete, cell cleavage occurs, forming a 2-cell embryo that includes a set of two centrioles, pericentrin, centrin, and γ-tubulin, components of the centrosome that play a critical role during syngamy. Pericentrin is involved in centrosome and spindle organization, centrin is important for centriole function and centrosome duplication, and γ-tubulin is mainly associated with PCM and the initiation of microtubule nucleation. Since the centrosome is crucial for normal chromosomal segregation at the first mitotic division, it seemed appropriate to perform centrosome assessment in the patient. To identify the human sperm centrosome, spermatozoa must be permeabilized and incubated with anti-centrin antibody followed by a secondary antibody. The specimen is then assessed under fluorescent microscopy, and the percentage of sperm cells exhibiting a centrosome is recorded (Fig. 27.6). In a normal sperm specimen, the percentage of sperm cells with a centrosome is expected to be between 60 and 80%. In this case, we detected a borderline normal presence of centrosome at 57%.

Transmission Electron Microscopy: To visually examine the ultrastructural aspect of the male gamete in this patient with faulty spermiogenesis, we performed transmission electron microscopy (TEM) to better assess sperm organelles such as the acrosome, nucleus, centrioles, and microtubular arrangement in flagella. The post-centrifugation sperm pellet was fixed by glutaraldehyde and sliced by ultramicrotome to 100 nm slices. Sections were then viewed by an electron microscope (JEOL-1400, JEOL USA, Inc., Peabody, MA, USA) with a magnification of 300,000×, where a 120-kV electron beam was transmitted through the specimen to

Fig. 27.6 Centrosome
Assessment on
globozoospermic
spermatozoa
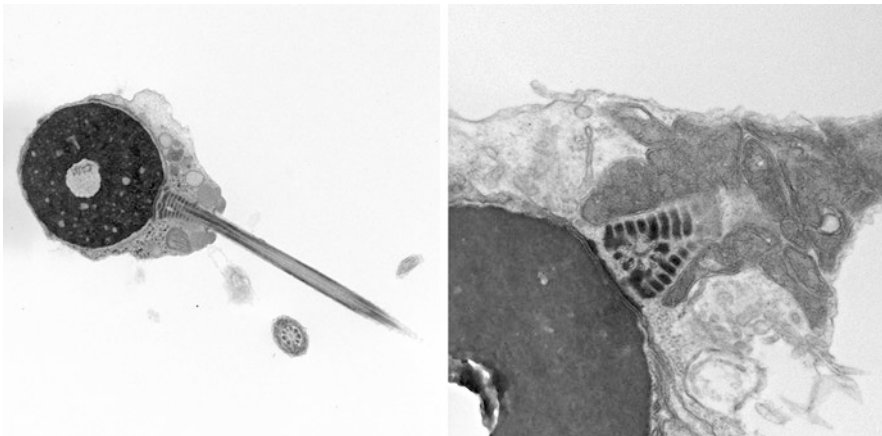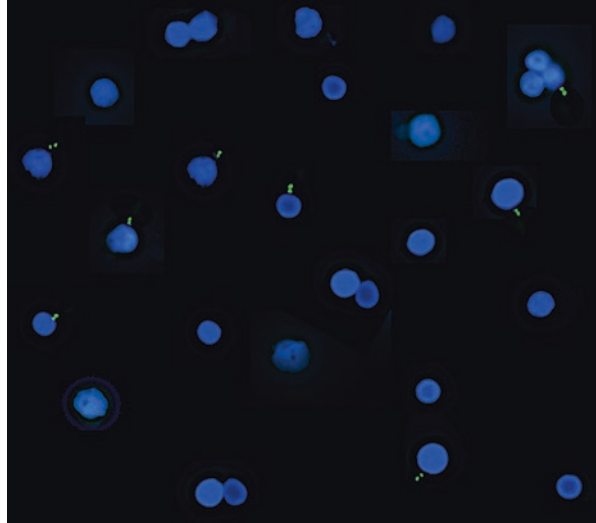
Fig. 27.7 Transmission electron micrograph of a globozoospermic specimen

produce a visual topography from the reflection of the electrons (Fig. 27.7). The
resulting images confirmed that all spermatozoa carried a round head, completely
lacking an acrosomal cap. The large majority (>80%) of the cells analyzed had a
cytoplasmic remnant containing residuals of the Golgi apparatus surrounding part
of the head and mainly located at the midpiece. Approximately 70% of the cells
analyzed had intranuclear vacuolizations and inclusions, with obvious abnormal
nuclear compaction. The proximal centriole with capitulum, when intercepted by
this section, appears visible and normal.

Genetic and Epigenetic Profiling: Recent literature has contributed to a greater
understanding of the genetic causes of infertility. With the identification of a

growing number of gene mutations specifically related to male factor infertility, we have also begun to develop a method for infertility screening by assessing the genome of the male patient [4]. The current availability of more thorough molecular genetic techniques, such as Next Generation Sequencing (NGS), has made it possible to assess the entire genome of the infertile male. It is also possible to constrain NGS to specific areas of interest, such as the exome, focusing on specific genetic markers associated with infertility. While genetic assessments have more commonly been carried out on peripheral blood, which can only identify uninheritable somatic mutations, we perform NGS on spermatozoa to detect germline mutations that may be passed onto offspring and may possibly be missed in somatic mutation analysis. To perform NGS, DNA must first be extracted and amplified, which can be achieved with the use of a commercially available kit. If the DNA is of adequate concentration and quality, it is submitted for sequencing. The resulting data can then be used to assess copy number variants (CNVs), which can be further annotated for the detection of gene mutations. Moreover, as it is not limited by the number of chromosomes that may be assessed, NGS can also be used for a more thorough evaluation of sperm aneuploidy. It is of paramount interest when assessing gene function to profile the individual epigenetically. This can be done by sequencing RNA, also by NGS, which offers insight into the expression of the gene of interest. Our assessment of this globozoospermic case using NGS was carried out by sequencing DNA extracted from spermatozoa, yielding an overall aneuploidy of 8.2%. We found a mutation on the *DPY19L* gene, which is recognized as a major cause of globozoospermia, as well as mutations on *SPATA16* and *PICK1*, which are also typical in globozoospermic men [5]. In addition, our NGS assessment revealed mutations on genes involved in spermiogenesis and embryo development, such as *PIWIL1*, *BSX*, and *NLRP5*. Our epigenetic analysis, carried out by RNAseq revealed that *DPY19L2* and *PICK1* were significantly overexpressed, confirming the malfunction of those two genes. Moreover, a significant underexpression of two other genes involved in oocyte activation by signaling calcium channel proteins (*AHNAK2*) and embryo development (*MMP14*) were also identified. Overall, these findings confirmed a genetic etiology of globozoospermia due to specific DNA sequencing mutations (*DPY19L, SPATA16, PICK1*), confirmed by gene expression profiling regarding an impaired spermatozoa oocyte activating potential (*AHNAK2*).

The results of the advanced sperm function tests unanimously showed that the spermatozoa were clearly impaired in their ability to successfully activate the oocyte. Therefore, we opted to treat the couple with in vitro insemination by ICSI using a proprietary assisted gamete activation (AGA) protocol targeted toward the male gamete as well as the oocyte, justified by the remarkable lack of PLCζ observed.

Superovulation Protocol: On cycle day (CD) 2, the female partner had an FSH of 3.82 IU, LH of 4.17 IU, E2 of 134.6 pg/mL, and P4 of 0.35 ng/mL. The female was superovulated with E2 patch priming, 50 mg clomid, 225 units of follitropin and 75 units of hMG, with BID GnRH antagonist. The patient was triggered with 4 mg of Lupron and 1500 IU human chorionic gonadotropin (hCG) on CD15, with an E2
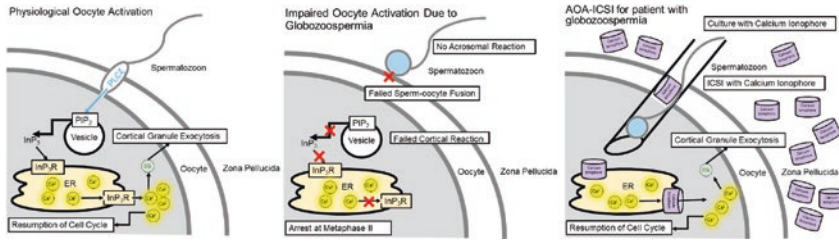
Fig. 27.8 Schematic of Oocyte Activation

of 3266 pg/mL, LH of 2.8 IU, and a lead follicle size of 22.5 mm. Her E2 the following day was 3845 pg/mL and her LH was 132 IU.

ICSI with AGA: A total of 14 oocytes were retrieved, of which 10 were mature. According to our proprietary protocol, prior to ICSI injection, ejaculated spermatozoa were briefly exposed to calcium ionophore in a drop on the ICSI dish. During the ICSI procedure, spermatozoa were aspirated individually from the drop containing calcium ionophore and immobilized in a separate PVP drop. Then, a small portion of calcium ionophore was aspirated into the micropipette and injected into the oocyte with the spermatozoa (Fig. 27.8). Post-ICSI oocytes were then exposed to calcium ionophore for a short period at 37°C, then washed and placed in IVF culture media.

Clinical Outcome: Fertilization was assessed 16–18 hours after ICSI. Four oocytes were successfully fertilized, confirmed by the appearance of two pronuclei and the extrusion of a second polar body. On day 3 post-ICSI, embryo cleavage was assessed. Two embryos (7-cell, 0% fragmentation; 8-cell, 7% fragmentation) were transferred. The other two embryos were maintained in culture, resulting in one arrested embryo and one average quality blastocyst that was cryopreserved. Approximately 2 weeks after embryo replacement, the patient's βhCG level was 596 mIU/mL. Two fetal yolk sacs were observed at 5 weeks and 5 days, and two fetal heart beats were observed 1 week later. At a gestational age of 38 weeks and 3 days, 2 healthy female offspring, weighing 2353 g and 2807 g, were born by elective caesarean section for malpresentation. No prenatal or postnatal complications were reported.

Discussion

An infertile couple where the female partner presents with a negative workup may imply that the cause of infertility resides with the male partner. Semen analysis is the primary tool to assess the male reproductive profile, however, it may not suffice in providing information on the functional capacity of the male gamete. In this couple, we utilized several sperm function assays to evaluate the fertilization capacity of the spermatozoon as well as its embryo developmental competence.

The protamine assay indicated an abnormal nuclear compaction and therefore a higher susceptibility of this individual's spermatozoa to be damaged by reactive oxidative species within the male genital tract. Therefore, this protamine deficiency may lead to high DNA fragmentation, which we assessed by TUNEL that resulted in a borderline abnormal sperm chromatin fragmentation.

Prior to advising the couple to proceed with IVF, we decided to assess the chromosomal profile of the male gamete in question, which fortunately resulted in only a borderline abnormal incidence of aneuploidy mainly characterized by autosomal and gonosomal disomies. This assay is important to measure the eventual contribution of the male partner to an increased incidence of embryo aneuploidy and higher pregnancy loss.

Another crucial aspect of the male gamete is the presence and integrity of the centrosome. Its presence in this particular individual is reassuring, as this indicates the ability of the spermatozoon to form the first mitotic spindle and ordain a correct segregation of the chromosome at the first cleavage division [3].

The ultrastructural analysis proved to be confirmatory in evidencing that a large majority of the cells had an absent acrosome, with almost nonexistent perinuclear theca, confirming abnormal compaction of the chromatin but also providing reassuring detail on the integrity of the midpiece with a normal structural appearance of the capitulum and the proximal centriole.

Needless to say, the most critical aspect is the assessment of the functional ability of the spermatozoon to activate the oocyte. A PLCζ assay confirmed that this labile protein, normally located in the perinuclear theca, was almost absent. The absence of this sperm-bound cytosolic factor undoubtedly proves the inability of the male gamete to activate an oocyte. The outcome of this assay represents the most important indication for assisted oocyte activation that has often been overlooked in the literature. Indeed, only about 20% of studies on this topic have carried out a functional male gamete assessment, such as the mouse oocyte activation test (MOAT) [1, 2].

In our genetic and epigenetic assessment, we identified a mutation of *DPY19L* by DNAseq, but when we epigenetically assessed the transcript by RNAseq, we found an additional imbalanced expression of *PICK1*. These are two of the most relevant genes involved in causing globozoospermia [5]. In addition, other genes (*PIWIL1, BSX, NLRP5*) involved in spermiogenesis and embryo development were imbalanced. Finally, significant underexpression of genes involved in oocyte activation by signaling calcium channel proteins (*AHNAK2*) and embryo development (*MMP14*) were identified. We therefore confirmed the genetic etiology of this man's condition using DNA and RNAseq, which can be used to properly counsel the couple.

On the basis of our assessment with different sperm function assays, the utilization of ICSI supported by AGA proved to be appropriate [2]. The adoption of this treatment has also proven to be safe in generating healthy offspring [1].

Overall, these additional tests on the male gamete provided insightful information on the globozoospermic diagnosis and prompted us to utilize the proper course of action which, in this particular case, was ICSI with assisted gamete activation to compensate for the lack of sperm acrosome and cytosolic sperm activating factor.

The utilization of advanced sperm function tests allowed this couple to achieve fertilization and successful embryo implantation, resulting in the live birth of two healthy baby girls.

References

1. Bonte D, Ferrer-Buitrago M, Dhaenens L, Popovic M, Thys V, De Croo I, et al. Assisted oocyte activation significantly increases fertilization and pregnancy outcome in patients with low and total failed fertilization after intracytoplasmic sperm injection: a 17-year retrospective study. Fertil Steril. 2019;112(2):266–74.
2. Neri QV, Lee B, Rosenwaks Z, Machaca K, Palermo GD. Understanding fertilization through intracytoplasmic sperm injection (ICSI). Cell Calcium. 2014;55(1):24–37.
3. Palermo GD, Colombero LT, Rosenwaks Z. The human sperm centrosome is responsible for normal syngamy and early embryonic development. Rev Reprod. 1997;2(1):19–27.
4. Cheung S, Parrella A, Rosenwaks Z, Palermo GD. Genetic and epigenetic profiling of the infertile male. PLoS One. 2019;14(3):e0214275.
5. Modarres P, Tanhaei S, Tavalaee M, Ghaedi K, Deemeh MR, Nasr-Esfahani MH. Assessment of DPY19L2 deletion in familial and non-familial individuals with globozoospermia and DPY19L2 genotyping. Int J Fertil Steril. 2016;10(2):196–207.

Chapter 28
Psychological Factors and Fertility Counseling

Elizabeth Grill

Case

John (43) and Julie (39) have been married for 5 years and have tried unsuccessfully to start a family for the past 2 years. Julie wanted to start trying to conceive 1 year before John who wished to wait until he was promoted at work. They tried to conceive on their own for 1 year before seeking help from a reproductive endocrinologist (RE). After receiving a combined factor diagnosis, they attempted 3 cycles of intrauterine insemination (IUI) and 3 cycles of in vitro fertilization (IVF). They became pregnant during the last IVF cycle but miscarried at 8 weeks. They plan to do another treatment cycle but their RE advised them to start thinking about alternative family building options. Prior to starting a fourth IVF cycle, they decide to talk with a mental health professional who specializes in couple's issues related to reproductive medicine.

The constant barrage of shots, blood tests, and surgical procedures left Julie feeling physically and emotionally exhausted. John found it difficult to connect with Julie as she became more withdrawn, depressed, anxious, lethargic, and uninterested in the things they both used to enjoy doing together. John complained that he no longer recognized Julie and feared that he would never get the person he knew back. Over time, this couple began to feel that the very foundation of their relationship was shaken as they were challenged to cope with stress and vulnerability, the loss of a dream, a sense of powerlessness, and feelings of guilt as well as blame.

While their marital and sexual relationship were strong at the beginning of treatment, the cumulative stresses of the infertility experience started to take a toll on their marital and sexual satisfaction. Throughout the course of treatment, this couple

E. Grill (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: eag2001@med.cornell.edu

reported sexual problems ranging from lack of desire, pleasure, and spontaneity to erectile dysfunction. Common feelings of infertility such as loss, anger, guilt, despair, depression, shame, and anxiety, began to overshadow intimate feelings of warmth, affection, and emotional connection so that sex became methodical, predictable, and unexciting. This added additional pressure for John who felt the responsibility of performing on demand (e.g., during ovulation, producing semen samples, etc.).

Their differing emotional reactions, communication styles and coping strategies left each of them feeling alone in the struggle, and the stresses resulting from multiple treatment failures put a strain on their personal and emotional well-being. Both felt a sense of profound loss and hopelessness from the unsuccessful IUI and IVF cycles and the miscarriage. They also feared the loss of a genetic tie to potential offspring as they contemplated donor gametes and adoption. They felt a sense of isolation from others and could not agree upon how much information they would share with family and friends and how often they would socialize and attend gatherings that involved children or the potential announcement of pregnancies. Both Julie and John reported feeling isolated, emotionally unsupported, depressed, and rejected by the other.

The case presented above illustrates the typical stressors and psychosocial complications of a couple struggling to conceive. This chapter will address an overview of the psychosocial interplay between infertility and relationship health and will provide a framework to providers about how to address these complex issues.

Discussion

Individuals and couples experience high levels of stress as they attempt to manage the physical, emotional, social, and financial concerns related to infertility and treatment. Difficulty conceiving is described as an emotional roller coaster and crisis that chips away at one's self-esteem, identity, relationships, and ability to cope [1]. Women undergoing fertility treatment characterize infertility as the most stressful experience of their lives [2] and On The Life Events Scale, the failure of IVF is rated equally to breast cancer, death of a family member, and worse than divorce [3].

While trying to conceive, patients no longer feel in control of their bodies or their life plan. Lives are put on hold, attempts to conceive become all consuming, and couples are beholden to treatment [4]. Trying to juggle medical appointments and medicine regimes with job responsibilities can increase pressure and put stress on careers. Not surprisingly, the most common reason why insured patients drop out of treatment is psychological burden resulting from infertility, including treatment [5].

The expectations and communication between a couple are also challenged. In some relationships, partners blame themselves or each other for infertility or medical diagnoses, resulting in anger that interferes with communication and sexual desire and functioning. Often times, one partner will harbor resentment toward the partner that wanted to wait longer to build a family. In another typical scenario, one

partner will blame the other partner's poor habits such as smoking, unhealthy eating, or lack of exercise for their problems.

Although there appear to be clear differences in the ways that men and women respond to infertility [6], research has shown that overall, both men and women with infertility are significantly more distressed than fertile couples [7, 8]. Women also experience greater emotional distress than men because they often assume more personal and social responsibility while managing more of the physical and emotional burden of treatment [4, 9].

Motherhood is seen by many women as a primary role in life and as an integral part of their femininity, gender identity, and sexuality. Women tend to assume that they are the cause of the infertility and begin (often fruitlessly) to search for a cause. They reproach themselves for past "misdeeds" and may even offer to leave their partners so as to free them to have children with another partner. Those who have difficulty getting pregnant or carrying a pregnancy may also feel like a failure as a human being. It is common for some infertile women to describe themselves as "defective" or "damaged goods." Clearly this self-perception can lead to depression, anxiety, and a loss of self-confidence and competence.

For many men, masculinity and fertility are deeply intertwined. If a male factor is diagnosed, self-esteem and self-image may also be negatively affected resulting in more anxiety, and physical symptoms as well as feelings of shame, loss, and poor self-esteem [7, 8]. An inability to impregnate his partner may strike heavily at a man's view of himself as "whole" and as virile and masculine. Women often have difficulty having empathy for their partners and may not understand the shame a man who associates potency with manliness may feel when he can't "make" his partner pregnant, or the anxiety and shame he reports feeling when forced to provide a sperm sample. In a recent study [10], 93% of men stated their well-being had been impacted by infertility and described it as "the most upsetting, dark and emasculating experience of my life." There is also less social acceptability for men to express these feelings as well as those of disappointment and grief to their partner. Their reluctance to share may also be caused by fear that they will only contribute further to the emotional distress that the couple already experiences.

For many women, thoughts about the infertility as well as the need to discuss it can sometimes feel obsessive. The partner, on the other hand, may feel overwhelmed with his partner's sadness and her desire to talk about the infertility constantly. He may either respond with calm, reason, and optimism in an effort to comfort his partner and be "the strong one" or he may begin to withdraw because he feels like a failure who can't fix the problem. If he begins to pull back, she may start to feel that her partner does not care about her distress or does not really want a child. It is typical for the woman to want her partner to be more emotional and for the man to want his partner to be more rational. Women tend to have more difficulty staying optimistic and react to the infertility by expressing themselves emotionally. She may begin to resent her partner's hopefulness, ability to function effectively at work, and distract himself from the problems. As a result, partners may begin to doubt the stability of the relationship and may withdraw from one another, feeling angry, hurt, and alone.

The universal theme of loss for those struggling to conceive is powerful. The grief is complicated because the loss experienced is not just for the longed-for child but also represents loss of self-worth, hope, identity, control, and loss of where they thought they would be at this point in their lives. Guilty feelings are also common and can further fuel a sense of profound loss and responsibility toward family and friends. The infertile couple may experience overwhelming guilt and sorrow about disappointing or denying grandparents-to-be or potential aunts and uncles. The couple may also be consumed with guilt about being unable to fulfill their duties and responsibilities related to "carrying on the family name" or providing an heir.

In circumstances when the couple cannot work through their differences, they may benefit from counseling that can help them improve communication, respect their different coping styles, and ultimately get them back to working out problems as a team. It is encouraging to note that few couples actually separate or divorce as a result of their infertility. Although the difficulties surrounding efforts to get pregnant or carry a child can be extremely stressful for most couples, many ultimately feel that the experience brought them closer together and led to the development of better coping skills.

The field of fertility counseling comprises an eclectic use of a variety of treatment modalities including but not limited to psychoeducational counseling, therapeutic counseling (e.g., cognitive behavioral), supportive counseling, grief counseling, and counseling that assists couples in decision making about treatment and next steps. The key is to match the right intervention at the right time to serve the emotional needs of the individual and couple. Table 28.1 provides a list of some of the goals and techniques of therapeutic intervention.

The form of counseling provided will be determined by the needs of the couple, the timing of treatment (e.g., initiation, treatment, resolution), the couples' level of distress, individual personality, and coping factors. The timing of interventions and assessments might be particularly important to their effectiveness since the issues facing the couple at each phase of treatment can differ and require different therapeutic approaches.

Psychoeducational counseling most commonly occurs before treatment begins but can also take place throughout the fertility journey as couples navigate treatment options at different stages. Psychoeducational counseling is aimed at reducing feelings of helplessness and the stress of treatment though preparation. Psychoeducation enhances patient control, addresses decision making and treatment options, and manages expectations.

Less distressed couples may benefit from written psychosocial information provided at key times in treatment or brief counseling that emphasizes education. For example, counselors have developed interventions tailored to specific challenges, such as coping with the 2-week waiting period before the pregnancy test and preparing couples for treatment [11, 12].

For those whose coping resources are inadequate and/or depleted, such interventions might not be sufficient and ongoing supportive or therapeutic counseling can be used to decrease psychological distress and improve relationship satisfaction for couples experiencing more moderate to severe levels of distress. One form of

Table 28.1 Therapeutic interventions and techniques

Therapeutic interventions	Examples of techniques
Psychoeducational counseling	Provide information about treatment to empower patients and to manage expectations
Supportive counseling	Improve patients' emotional health and well-being through empathy, validation of feelings, and normalizing distress
Improve communication with doctor/staff/bosses/friends/family members	Role play typical interactions. Assertiveness training. Boundary setting
Reduce the stress and demands of fertility treatment	Relaxation techniques, mindfulness, cognitive behavioral strategies
Help make informed decisions about family building options or ending treatment	Enhance problem solving skills
Improve couple communication	Acknowledge different coping styles, learn to fight fairly, teach empathic listening skills
Improve intimacy and sexual relationship	Non procreative sex, dispute male/female myths, date night without fertility discussion, devote time to activities and interests that they enjoy together
Promote healthy coping strategies	Replace bad habits with positive coping strategies like exercise, healthy eating habits, relaxation techniques
Grief counseling	Introduce rituals that validate loss and help with acceptance and resolution
Encourage patients to make meaning of the infertility experience	Journaling, resilience training
Improve well-being	Self-care strategies, positive coping, restoring hope
Help set boundaries with friends and family members	Role play conversations, develop self-protective strategies
Understand social, cultural, and religious factors that contextualize infertility and treatment	Ask questions to become culturally competent and respectful, consult with clergy, healers, family members, etc. (with permission)

therapeutic counseling is cognitive behavioral therapy (CBT). There is overwhelming evidence that CBT is equivalent to antidepressant medication in the treatment of mild-to-moderate depression and more recent research indicates that it is effective in the treatment of severe depression as well [13]. This short-term form of therapy teaches individuals to recognize and challenge negative self-defeating thoughts and irrational beliefs about themselves, their environment and their future. Table 28.2 illustrates ways to restructure typical negative thoughts couples have about their fertility.

Longer-term therapeutic and grief counseling can be used when psychological stressors are more severe or after an unsuccessful fertility treatment cycle when stress is greatest. Grief counseling can occur at any point throughout the fertility journey. Couples can process frustration about starting later than desired, sadness about the number of eggs/embryos yielded or unsuccessful ART attempts. Couples may need to mourn miscarriages, selective reduction or termination. If couples decide to pursue third party reproduction or adoption, they will need to grieve the

Table 28.2 Cognitive restructuring

The next time you think …	Stop and replace it with…
"I waited too long"	"I wasn't ready before. Now my partner and I are in a better position both financially and emotionally"
"I'll never have a baby"	"I can't predict the future. I haven't had success yet, but my doctors are optimistic and I am doing everything that I can to pursue treatment"
"I can't do anything right, even get pregnant"	"I've had many successes in my life. I'm doing everything I can to optimize my chances but some things are out of my control"

loss associated with giving up a genetic connection. Ultimately, if couples end treatment and remain childless, they will need to process despair and make meaning of their fertility struggle.

The purposes of this chapter are to provide a general framework for understanding the complex struggles that couples face along the family building journey and to provide direction to providers about how to assess and treat these complicated issues. Mental health professionals trained in the field of reproductive medicine can intervene on several different therapeutic levels by providing patient education, supportive and grief counseling, and helping patients with treatment decisions. Given the reported levels of distress experienced by infertility couples, medical treatment should be in conjunction with counseling, emphasizing the importance of emotional health, and well-being in couples struggling to build their families.

References

1. Andrews F, Abbey A, Halman L. Is fertility-problem stress different? The dynamics of stress of fertile and infertile couples. Fertil Steril. 1992;57(6):1247–53.
2. Whiteford LM, Gonzalez L. Stigma: the hidden burden of infertility. Soc Sci Med. 1995;40(1):27–36.
3. Van den Broeck UE, Pasch L, Katz P, Millstein S, D'Hooghe T, Demyttenaere K. Longitudinal follow-up of the intrusiveness of the infertility experience: predictors and gender differences. Fertil Steril. 2010;4:S65.
4. Pasch LA, Holley SR, Bleil ME, Shebab D, Katz PP, Adler NE. Addressing the needs of fertility treatment patients and their partners: are they informed of and do they receive mental health services. Fertil Steril. 2016;106(1):209–15.
5. Gameiro S, Boivin J, Peronace L, Verhaak CM. Why do patients discontinue fertility treatment? A systematic review of reasons and predictors of discontinuation in fertility treatment. Hum Reprod Update. 2012;18(6):652–69.
6. Bayley T, Slade P, Lashen H. Relationships between attachment, appraisal, coping and adjustment in men and women experiencing infertility concerns. Hum Reprod. 2009;24(11):2827–37.
7. Kedem P, Mikulincer M, Nathanson YE, Bartoov B. Psychological aspects of male infertility. Br J Med Psychol. 1990;63(Pt 1):73–80.
8. Nachtigal RD, Becker G, Wozny M. The effect of gender-specific diagnosis on men's and women's response to infertility. Fertil Steril. 1992;57:113.

9. Wright J, Duchesne C, Sabourin S, Bissonnette F, Benoit J, Girard Y. Psychosocial distress and infertility: men and women respond differently. Fertil Steril. 1991;55(1):100–8.
10. Hanna E, Gough B. Emoting infertility online: a qualitative analysis of men's forum posts. Health (London). 2016;20(4):363–82.
11. Lancaster D, Boivin J. A feasibility study of a brief coping intervention (PRICI) for the waiting period before a pregnancy test during fertility treatment. Hum Reprod. 2008;23(10):2299–307.
12. Klonoff-Cohen H, Chu E, Natarajan L, Sieber W. A prospective study of stress among women undergoing in vitro fertilization or gamete intrafallopian transfer. Fertil Steril. 2001;76(4):675–87.
13. Parikh S, Zindel S, Segal S, Grigoriadis S, Ravindran A, Kennedy H, et al. Canadian network for mood and anxiety treatments (CANMAT) clinical guidelines for the management of major depressive disorder in adults. II. Psychotherapy alone or in combination with antidepressant medication. J Affect Disord. 2009;117(1):S15–25.

Chapter 29
Intrauterine Insemination

Phillip Romanski, Pietro Bortoletto, and Pak H. Chung

Case

A 29-year-old nulligravida with a 15-month history of infertility presented for evaluation. She reported menarche at age 14 with irregular cycles of 90–120 days in length. As a teenager, she suffered from acne and difficulty in managing her weight despite diet and exercise. At age 22 she was diagnosed with polycystic ovary syndrome (PCOS) and was started on a combined oral contraceptive pill which improved the regularity of her menstrual cycle and her acne. She has now been married for 3 years and has been attempting to conceive for the past 15 months. Since stopping her oral contraceptive pill 15 months ago, she has menstruated only four times.

Her past medical history is unremarkable aside from PCOS, class II obesity, and acne. She has no surgical history and uses topical salicylic acid. Her family history is significant for hypertension and coronary artery disease. She is a non-smoker, denies illicit drug use, and uses alcohol socially. Her partner is a 31-year-old healthy male who has never achieved a conception in the past. He does not have any relevant medical or surgical history and does not take medications. Neither does he smoke or use illicit drugs. He consumes 6–10 alcoholic beverages per week.

As part of her evaluation a pelvic vaginal ultrasound was performed and revealed a normal, anteverted uterus with a 6.5 mm endometrial thickness. Both of her ovaries were notable for numerous small antral follicles, consistent with her PCOS

P. Romanski · P. Bortoletto
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA

P. H. Chung (✉)
Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: pakchu@med.cornell.edu

diagnosis. Laboratory evaluation revealed a day 3 follicle stimulating hormone (FSH) of 6.2 mIU/mL, luteinizing hormone (LH) of 8.8 mIU/mL, and estradiol (E2) of 67 pg/mL. An anti-Müllerian hormone returned at 8.1 ng/mL. Her partner's semen analysis with strict Kruger criteria was notable for a semen volume of 1.5 mL, concentration of 14 million/mL, motility of 34%, and normal morphology of 4%.

Given her polycystic ovary syndrome with oligomenorrhea and her partner's oligoasthenospermia, she was recommended to undergo ovulation induction with intrauterine insemination (IUI), while the husband waited for a urologist consultation. She was treated with letrozole 2.5 mg, an aromatase inhibitor, starting on day 3–7 of her menstrual cycle. A single dominant follicle was seen on ultrasound on day 13. She received a recombinant hCG ovulatory trigger followed by intrauterine insemination 24 h later. She conceived immediately after the first insemination and a singleton and viable intrauterine pregnancy was detected on transvaginal ultrasound at 7 weeks of gestation. She was referred to an obstetrician for prenatal care.

Discussion

Intrauterine insemination (IUI) is a minimally invasive technique that allows for washed sperm to be deposited directly into the uterine cavity via a transcervical catheter. IUI is commonly used in the treatment of unexplained infertility, in the presence of mild endometriosis, mild male factor, or a cervical factor. Because IUI can bypass the need for penetrative intercourse or a male partner, it can also be utilized for third-party reproduction and couples who are unable to have penetrative intercourse. Additionally, IUI can be utilized as a risk reducing reproductive option for sero-discordant couples with HIV when paired with specialized sperm preparation techniques to rid the semen of viral particles.

To proceed with IUI, several important conditions must be met. First, patency of at least one fallopian tube must be confirmed, either via hysterosalpingography or other techniques involving sonographic contrast to evaluate tubal patency. Second, there must be no documented evidence of pelvic infection or cervicitis in the peri-insemination period. Cervical cultures have to be negative prior to IUI. Third, a semen analysis should confirm the presence of motile sperm in the ejaculate. Severe oligospermia is a relative contra-indication to IUI and offering IUI should be considered in the context of each individual patient.

Intrauterine insemination is a relatively low-risk procedure. Intra-procedure concerns include patient discomfort and bleeding from the passage of the catheter through the cervix. Upper genital tract infection is a rare potential complication of IUI occurring in approximately 1.83 per 1000 women [1]. Common infectious organisms include lower genital tract bacteria such as Escherichia coli and anaerobic bacteria. Given the low rate of pelvic infection following IUI, routine antibiotic prophylaxis is not recommended.

In its simplest form, IUI can be performed around the time of ovulation in a natural menstrual cycle to overcome a mild male factor, cervical factor, or for third-party reproduction. In these instances where ovarian reserve is optimal and the patient is ovulatory, ovulation induction is not necessary and has not been shown to increase pregnancy outcomes but may increase the risk of multiple gestation. In patients with unexplained infertility or mild endometriosis, pregnancy, and live birth outcomes are improved when ovulation induction is paired with IUI. A randomized controlled trial of patients with unexplained infertility randomized to expectant management, natural cycle with IUI, and clomiphene citrate with timed intercourse did not demonstrate a benefit to natural cycle with IUI compared to expectant management (23 vs. 17%, $p = 0.11$) [2]. However, this study did not include a treatment arm evaluating ovulation induction in combination with IUI. In a randomized trial by Guzick et al., patients with unexplained or mild endometriosis infertility were randomized to one of four arms: natural cycle with intracervical insemination (ICI), which served as the control arm, natural cycle with IUI, ovulation induction with ICI, or ovulation induction with IUI [3]. Pregnancy rates in the natural cycle with ICI group were 2% per cycle and 10% cumulatively. Pregnancy rates were significantly higher in both the natural cycle with IUI group (5% per cycle, $p = 0.01$; 18% cumulatively, $p = 0.003$) and in the ovulation induction with IUI group (9% per cycle, $p < 0.001$; 33% cumulatively, $p < 0.001$). Additional support for pairing ovulation induction with IUI in patients with unexplained infertility was demonstrated in a study by Reindollar et al. in which per cycle live birth rates were reported to be 7.1% per cycle with a cumulative live birth rate of 19.4%, which is higher than was is typically observed in patients who are treated with expectant management or with ovulation induction alone [4].

In patients with infertility due to anovulation, intrauterine insemination is not routinely recommended due to a lack of clear benefit [5]. However, a portion of these patients will also have a co-existing mild male factor, cervical factor, or mild endometriosis associated infertility which could benefit from the addition of an IUI procedure. Therefore, clinicians may choose to pair IUI with ovulation induction in these patients in order to overcome any of these known or unknown co-existing infertility factors.

Regardless of adjunctive oral or even injectable agents, the optimal time to perform an IUI is based around the day of ovulation. After ovulation, an oocyte remains viable for 12–24 h, however sperm survives in the female reproductive tract for 2–3 days. Patients can either monitor for their luteinizing hormone (LH) surge using urine detection kits or they can be given an ovulatory trigger when transvaginal ultrasound examination shows a mature follicle. The benefit of administering an ovulatory trigger once the dominant follicle has reached a size that is expected to ovulate a mature oocyte is that it limits the number of patient visits and it allows for a more predictable scheduling pattern for when the IUI procedure will occur. Studies which have evaluated the time from ovulatory trigger to IUI procedure have shown comparable results whether the IUI was performed on the day of the trigger, 24 h after, or 36 h after the trigger was administered [6, 7].

Both fresh and frozen semen can be utilized for the purposes of IUI. Frozen donor semen is often used by single women or those in same-sex relationships. In cases where a male partner is producing a fresh semen specimen, 2–3 days of abstinence prior to production is generally recommended. Both fresh and cryopreserved semen undergo processing to rid the ejaculate of prostatic secretions and cellular debris. Sperm processing, either via the "swim-up" method or "density gradient centrifugation" serves to maximize the concentration of motile sperm for insemination. A Cochrane Database Review of both sperm preparation techniques found insufficient evidence that any single technique provided a statistically significant increase in pregnancy rates [8]. An additional benefit to semen preparation is the ability to "wash" the semen of HIV positive men, effectively ridding the sperm of viral particles, and providing a risk reducing reproductive option for sero-discordant couples [9].

The number of IUI cycles to attempt prior to escalating treatment to other ART techniques is an area of debate. In the study of a prospective cohort of over 400 couples across eight institutions who were attempting to conceive, a benefit was seen in up to 3 cycles of IUI. No additional benefit was seen for couples using four or more IUI cycles to conceive (HR: 1.0, 95%: 0.4–2.6) [10]. In contrast, a European multicenter, retrospective cohort of over 700 couples with male, cervical or unexplained subfertility found that mean ongoing pregnancy rates (5.6%) did not substantially differ from the seventh, eighth or ninth IUI attempt (5.1, 6.7, and 4.6%) [11]. The American Society of Reproductive Medicine Practice Committee recommended that couples with unexplained infertility undergo 3–4 cycles of ovarian stimulation and IUI with oral agents prior to escalation to IVF [12]. For patients with male factor or anovulation, clinical context and patient preference should be taken into account when deciding how many IUI cycles to undergo before moving to IVF.

In summary, IUI is an important, common, and low-risk infertility treatment technique which is useful in the early management of most types of infertility as well as in third-party reproduction. Sperm preparation techniques serve to improve the quality of the semen specimen and the insemination catheter circumvents cervical and mild male factor causes of infertility by depositing the sperm directly inside the uterine cavity. The addition of ovulation induction agents, such as clomiphene citrate or letrozole, may improve cycle outcomes for some patients. Patients who are likely to have a successful outcome after an IUI procedure will most often become pregnant after 3–6 attempts. For patients who do not conceive, more aggressive treatment options, i.e. IVF should be considered.

References

1. Sacks PC, Simon JA. Infectious complications of intrauterine insemination: a case report and literature review. Int J Fertil. 1991;36(6):331–9.
2. Bhattacharya S, Harrild K, Mollison J, Wordsworth S, Tay C, Harrold A, et al. Clomifene citrate or unstimulated intrauterine insemination compared with expectant management for unexplained infertility: pragmatic randomised controlled trial. BMJ. 2008;337:a716.
3. Guzick DS, Carson SA, Coutifaris C, Overstreet JW, Factor-Litvak P, Steinkampf MP, et al. Efficacy of superovulation and intrauterine insemination in the treatment of infertility. N Engl J Med. 1999;340(3):177–83.
4. Reindollar RH, Regan MM, Neumann PJ, Levine B-S, Thornton KL, Alper MM, 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.
5. Weiss NS, Nahuis MJ, Bordewijk E, Oosterhuis JE, Smeenk JM, Hoek A, et al. Gonadotrophins versus clomifene citrate with or without intrauterine insemination in women with normogonadotropic anovulation and clomifene failure (M-OVIN): a randomised, two-by-two factorial trial. Lancet. 2018;391(10122):758–65.
6. Rahman SM, Karmakar D, Malhotra N, Kumar S. Timing of intrauterine insemination: an attempt to unravel the enigma. Arch Gynecol Obstet. 2011;284(4):1023–7.
7. Vichinsartvichai P, Traipak K, Manolertthewan C. Performing IUI simultaneously with hCG administration does not compromise pregnancy rate: a retrospective cohort study. J Reprod Infertil. 2018;19(1):26–31.
8. Boomsma CM, Heineman MJ, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. Cochrane Database Syst Rev. 2007;(4):CD004507.
9. Zafer M, Horvath H, Mmeje O, van der Poel S, Semprini AE, Rutherford G, et al. Effectiveness of semen washing to prevent human immunodeficiency virus (HIV) transmission and assist pregnancy in HIV-discordant couples: a systematic review and meta-analysis. Fertil Steril. 2016;105(3):645–55.
10. Smith JF, Eisenberg ML, Millstein SG, Nachtigall RD, Sadetsky N, Cedars MI, et al. Fertility treatments and outcomes among couples seeking fertility care: data from a prospective fertility cohort in the United States. Fertil Steril. 2011;95(1):79–84.
11. Custers IM, Steures P, Hompes P, Flierman P, van Kasteren Y, van Dop PA, et al. Intrauterine insemination: how many cycles should we perform? Hum Reprod. 2008;23(4):885–8.
12. Practice Committee of the American Society for Reproductive Medicine. Evidence-based treatments for couples with unexplained infertility: a guideline. Fertil Steril. 2020;113(2):305–22.

Chapter 30
Assisted Reproductive Technology

Nigel Pereira and Zev Rosenwaks

Case

A 35-year-old gravida 2 para 0 woman presents to the office for the evaluation of 18-month history of involuntary infertility. Her anti-Mullerian hormone (AMH) and cycle day 2/3 follicle-stimulating hormone (FSH) levels 2 years prior to presentation were 0.04 ng/mL and 19.8 mIU/mL, respectively. Her prior two conceptions occurred naturally but resulted in biochemical pregnancies. Her partner is a 33-year-old gentleman with no pertinent urologic history, except for isolated teratozoospermia on semen analysis. She underwent three IVF cycles at another institution, yielding a single cryopreserved (2pn) zygote. Her current evaluation was significant for an AMH of 0.08 ng/mL and cycle day 2 FSH of 48.5 mIU/mL and LH of 18 mIU/mL. How does a physician approach this clinical presentation?

Background

The diagnosis and treatment of most medical conditions are individual-based i.e., a single individual undergoes treatment, and successful outcome hinges on the patient-physician relationship and that individual's compliance to treatment. In contrast, infertility treatment involves a couple whose general and reproductive health

N. Pereira
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: nip9060@med.cornell.edu

Z. Rosenwaks (✉)
Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: zrosenw@med.cornell.edu

is often evaluated by multiple medical providers in parallel. Optimal fertility treatment requires collaboration within the couple, between the couple and medical providers, sometimes the involvement of an embryology or andrology laboratory. The management of infertility has rapidly evolved over the past four decades, and in some instances, there has been an accelerated adoption of certain fertility treatments despite limited clinical evidence. In this chapter, we review the epidemiology and diagnostic workup of female infertility. We specifically focus on Assisted Reproductive Technology (ART) as a therapeutic strategy for infertility. We also present the clinical outcomes associated with ART and also briefly appraise their safety.

Epidemiology

Infertility is commonly defined as the failure to conceive after 1 year of unprotected intercourse and is thought to affect approximately 15% of reproductive age couples worldwide. Human reproductive efficiency is relatively inefficient when compared to other species, including nonhuman primates. Reproductive efficiency in normally fertile couples is estimated at approximately 20% per menstrual cycle, which suggests that 85% of couples should conceive within 1 year. Therefore, a couple should undergo a fertility evaluation once the 1-year threshold has been met. However, a workup can be initiated sooner in women >35 years or age or those with pertinent medical and/or gynecologic findings. It is important to note that many couples undergoing infertility treatment may be sub-fertile, and not truly sterile. Therefore, a proportion of sub-fertile couples may conceive without any intervention.

Evaluation

The initial evaluation of infertility begins with a comprehensive history and physical examination of the female partner. For the purpose of this chapter, we will focus on the female workup. However, the male partner may require a complete urologic evaluation based on clinical history or abnormal semen analyses. A female fertility evaluation should include the following:

(a) Thorough developmental, medical, surgical, family, social, and sexual history. Surgical procedures such as ovarian cystectomy, hernia repairs, or pelvic surgery can impair fertility or ovarian reserve, and should therefore be reviewed. Any family history, particularly parental history of infertility or fertility treatments should be elicited. The social history should comprise a thorough review of tobacco or alcohol consumption, as well as use of recreational drugs. Eliciting history of any prior sexually transmitted infections, pelvic infections, pregnan-

cies with the current or previous partners, and incorrect patterns of timing inter-course generally comprises the sexual history. Given the potential adverse effects that many medications can have on fertility, all medications used by a patient, including dosage and route of administration should be noted in detail. Furthermore, any occupational exposure to pesticides, herbicides, radiation or industrial solvents should be investigated.

(b) Meticulous physical examination includes an assessment of body habitus, breast development, external genital examination, and an internal bimanual pelvic examination.

(c) Uterine cavity and tubal patency evaluation.

(d) Ovulatory status based on menstrual cycle regularity or measurement of a mid-luteal serum progesterone >3 ng/mL. In anovulatory women, serum prolactin and thyroid-stimulating hormone should also be measured.

(e) Ovarian reserve testing, which includes antral follicle counts via transvaginal ultrasonography, or measurements of anti-Mullerian hormone (AMH) and cycle day 2/3 follicle-stimulating hormone (FSH).

(f) Semen analysis with a concentration of 15 million sperm per milliliter, >40% motility, and at least 4% normal morphology by strict Kruger criteria. Given the inherent biological fluctuations between semen samples, a minimum of two samples should be examined.

(g) Laparoscopy may aid in the assessment of endometriosis, especially in women with a history of dysmenorrhea, pelvic pain, or endometriomas. While most data suggest that laparoscopy is not strictly necessary for infertility evaluation, each physician should carefully assess the benefits versus risks on an individual patient basis.

Diagnostic methods such as post-coital tests and endometrial biopsies were widely utilized in the evaluation of infertility. However, several large-scale studies have demonstrated that these methods lack accuracy and reproducibility, and often fail to distinguish between infertile and fertile women.

Couples can be classified into one of four categories based on the initial workup: female factor (~35% of cases), male factor (~30% of cases), and combined female and male factor (~20%). Couples who do not have a clear cause of infertility comprise the remaining 15% of cases in a category called unexplained infertility, which by definition, is a diagnosis of exclusion. Some large retrospective studies have reported that unexplained infertility may comprise up to 30% of all infertility cases; however, the incidence of these cases depends on the criteria used to define unexplained infertility. Given that there is no universal consensus as to which exact tests constitute a standard fertility evaluation, and no defined universal protocols for the diagnosis of unexplained infertility, the prevalence of unexplained infertility varies largely in the medical literature. The categorization of infertility types also aids in expediting treatment options, given that the likelihood of achieving a live birth without treatment decreases with increasing age of the female as well as duration of infertility.

Treatment

Non-ART Treatment

Lifestyle interventions to improve fertility include smoking cessation, reduction in caffeine and alcohol consumption, and normalization of body mass index (BMI). Changes in dietary habits have been shown to impact both ovulation rate and semen parameters. These changes include consumption of food with low-glycemic index and higher protein, especially from vegetable sources. The options for active management of infertility include ovulation induction with various oral and injectable pharmacologic agents, with or without intra-uterine insemination (IUI), and ART. We will focus our subsequent discussion on ART using autologous oocytes, i.e., non-donor oocytes.

ART

The Centers for Disease Control and Prevention (CDC) defines ART as any fertility treatment in which either oocytes or embryos are handled. Based on this definition, ART does not include treatments like IUI in which only sperm is handled or ovulation induction where multiple oocytes are stimulated without oocyte retrieval. ART includes treatments such as in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT), and zygote intrafallopian transfer (ZIFT), with IVF accounting for approximately 99% of all ART procedures. Contemporary IVF treatment involves ovarian stimulation using pharmacologic agents, transvaginal oocyte retrieval, fertilization of oocytes, culture of embryos, and embryo transfer (Fig. 30.1).
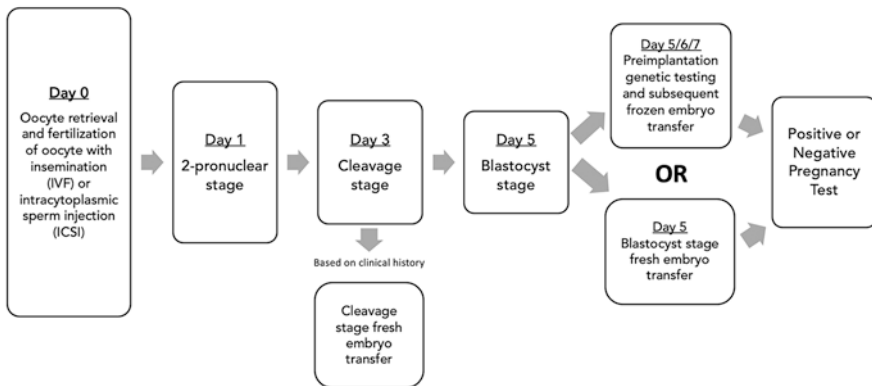
Fig. 30.1 Summary of contemporary IVF treatment

The International Committee for Monitoring Assisted Reproductive Technologies (ICMART) reported an estimated 1,929,905 ART cycles in 2014 from 2746 ART centers in 76 different countries. These ART cycles resulted in the birth of 439,039 babies during the reporting period. In the United States (U.S.) alone, a total of 203,119 ART cycles were performed in 456 U.S. fertility clinics in 2018. These cycles resulted in 73,831 live births. ART contributed to 2.0% of all infants born in the United States in 2018.

Indications for ART

Louise Brown, the first IVF baby in 1978, was born to a mother who underwent laparoscopic oocyte retrieval during the natural menstrual cycle and had a history of severe tubal disease requiring at least two prior laparotomies. Since then, at least nine million babies have been born worldwide using IVF for different infertility indications. The ART Fertility Clinic and National Summary Report from the CDC lists the following indications for all ART cycles in the U.S. during 2019: diminished ovarian reserve or ovulatory dysfunction (29%), male factor (27%), unexplained infertility (27%), tubal factor (10%), and endometriosis (7%).

Accelerated Utilization of ART

Efficient ovarian stimulation protocols, standardization of a simple outpatient oocyte retrieval technique, and successful laboratory techniques, including intracytoplasmic sperm injection (ICSI) and vitrification have often been recognized as the main reasons for the acceleration of ART utilization.

The success of IVF depends significantly on an individualized approach to ovarian stimulation (Fig. 30.2).

An efficacious ovarian stimulation protocol maximizes follicular development, improves oocyte and embryo quality, increases implantation and live birth rates, and minimizes the risks of complications such as ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies. The stimulation of multiple follicles with gonadotropins is associated with supraphysiologic estradiol (E2) levels, often inducing a premature luteinizing hormone (LH) surge, which historically was associated with IVF cycle cancelation. To circumvent the problem of a premature LH surge and/or ovulation in IVF cycles, co-administration of gonadotropin-releasing hormone agonists (GnRH-a) was introduced in the late 1980s. However, GnRH-a protocols were associated with higher rates of side effects, including mood changes, hot flushes, severe headaches, short-term memory loss, and OHSS. These shortcomings of GnRH-a protocols paved the way for potent gonadotropin-releasing hormone antagonists (GnRH-ant) in the early 1990s. GnRH-ant competitively binds to the GnRH
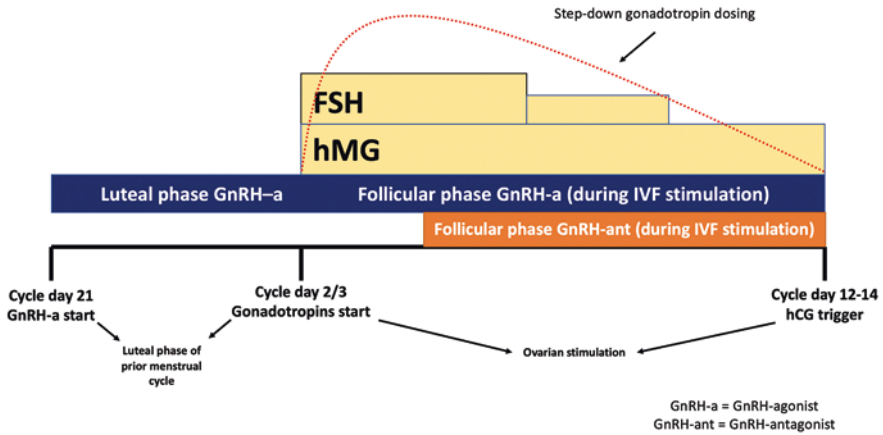
Fig. 30.2 Summary of ovarian stimulation for IVF. Please note that ovarian stimulation protocols can be GnRH-a or GnRH-ant based

receptor and suppresses LH secretion in a dose-dependent manner. Thus, GnRH-ant can be initiated earlier in the follicular phase, rendering shorter and more patient-friendly ovarian stimulation cycles. We prefer to begin antagonist administration when estradiol level is above 200 pg/mL or the most advanced follicle reaches 12–13 mm in average diameter. Furthermore, GnRH-ant protocols are associated with lower risks of OHSS. Thus, the favorable side effect profile associated with GnRH-ant protocols, without compromising IVF success rates, has resulted in its accelerated adoption in contemporary ART practice.

Oocyte retrieval was performed via laparoscopy in the very initial stages of ART. However, the need for general anesthesia, operating room setting, and longer procedural time associated with laparoscopic oocyte retrievals limited its application. Today, transvaginal oocyte retrieval is considered the standard of care across ART centers worldwide. This technique involves the use of a high-frequency transvaginal ultrasonographic transducer laden with a needle sheath. A 30 cm, 16 G, single-lumen or double-lumen aspiration needle is used to puncture the ovarian follicles using the needle sheath as a guide, with a constant pressure of 80–100 mmHg, which assists in the aspiration of follicular fluid.

ICSI is a procedure that involves the injection of a single spermatozoon into the cytoplasm of an oocyte. ICSI bypasses both the zona pellucida and sperm defects in the male gamete that compromises its ability to fertilize. The implementation of ICSI in the past three decades has made it possible to overcome severe male factor infertility and fertilization defects that would have been deemed unachievable previously. In the United States, ICSI rates have increased from 36.4% in 1996 to 76.2% in 2012.

ART Success Rates

Using data from all reporting ART clinics in the U.S. in the year 2018, the Society for Assisted Reproductive Technology (SART) reported 42,460 IVF cycle starts in women <35 years, 26,642 IVF cycle starts in women 35–37 years, 25,430 IVF cycle starts in women 38–40 years, 13,345 IVF cycle starts in women 41–42 years, and 9507 IVF cycle starts in women >42 years of age. The corresponding live birth rates in these groups were 44.6%, 31.5%, 19.9%, 9.7%, and 2.9%, respectively. Approximately 93–96% of all ART-conceived babies were singletons. This is predominantly due to the utilization of elective single embryo transfers (SET). The national SET rate among all ART cycles was 74.1% in women <35 year, 72.8 in women 35–37 years, and 66.4% in women >37 years of age. Global trends also suggest increased SET rates from 30.0% in 2010 to 40.0% in 2014. The accelerated trend in SET cycles has occurred due to the preferential transfer of blastocyst-stage embryos over cleavage-stage embryos. A recent meta-analysis of 32 randomized-controlled trials (RCTs) including 5821 couples reported higher odds of live birth (1.27, confidence interval 1.06–1.51) with the blastocyst-stage transfer when compared to cleavage-stage transfer, though this evidence was of low quality.

Recent Trends in ART

The past two decades has witnessed accelerated adoption of certain aspects of ART despite limited evidence. For example, even though ICSI is typically used for the treatment of male factor infertility, a surveillance study based on U.S. data found that ICSI use in non-male factor infertility increased from 15.4% to 66.9% between 1996 and 2012. More recent data suggest ICSI utilization rates of close to 80% even though 30% of IVF cases are for male factor infertility. These trends are not supported by multiple clinical studies showing no increase in fertilization or live birth rates with the use of ICSI for non-male factor infertility. Similarly, better vitrification techniques and improvements in culture media has resulted in the proliferation of frozen-thawed embryo transfer (FET) cycles and preimplantation genetic testing (PGT) cycles. U.S. data indicate an 82.5% increase in FET cycles between 2006 and 2012, while the number of fresh embryo transfers has increased by only 3.1%. These data indicate that many IVF centers have preferentially shifted toward FET cycles. The theorized benefits of FET over fresh embryo transfer have been propagated by the idea that the former provides a better implantation environment. However, recent multicenter randomized-controlled trials (RCTs) have highlighted that FET-associated improvements in pregnancy and perinatal outcomes are observed in the context of robust or exaggerated ovarian stimulation, such as women with polycystic ovarian syndrome (PCOS), and not in all women undergoing IVF. Finally, PGT-A has been heralded as a universal screening test for all women

undergoing IVF due to studies demonstrating higher SET and live birth rates after aneuploidy testing. In contrast, recent RCTs have failed to reproduce these results, raising important questions about patient selection, publication bias and testing platforms in the initial studies.

Safety of ART

Transfer of multiple embryos and multiple pregnancies contributed to the initial adverse obstetric and perinatal outcomes associated with ART. However, the increasing utilization of SET has resulted in lower multiple pregnancy rates, thereby decreasing obstetric and perinatal risks. Yet, the safety of ART, specifically ICSI, has been questioned given that the fertilizing spermatozoon is selected arbitrarily, and it neither binds to the zona pellucida nor fuses with oolemma. Thus far, studies of ICSI children have provided sufficient information to reassure these qualms. Population based studies have suggested a modest increase in small for gestational age babies (SGA), low birthweight (LBW), and preterm birth (PTB) with fresh embryo transfer and large-for-gestational age (LGA) babies and pre-eclampsia with FET.

Discussion

The patient in the current clinical case has poor ovarian reserve as exemplified by her ovarian reserve markers. Women with low ovarian reserve constitute a large number of IVF cases in the U.S.; however, treatment of women with poor ovarian reserve may not be straightforward. Women with high cycle day 2/3 FSH or low AMH often do not respond well to ovarian stimulation and/or may be at high risk of IVF cycle cancelation prior to oocyte retrieval. Furthermore, oocyte yield can be low even if oocyte retrieval is accomplished in such patients. Therefore, many clinics may not even consider ovarian stimulation for IVF in women with poor ovarian reserve and may encourage them to pursue treatment using donor oocytes.

The above patient presented to us with a history of elevated FSH and exceedingly low AMH levels. Due to poor ovarian reserve, she underwent three IVF cycles in another institution, together yielding a single cryopreserved 2 PN zygote. Given her high basal FSH level (>40 mIU/mL) and an LH level of 18 mIU/mL, she underwent a single gonadotropin stimulated cycle after luteal estradiol suppression which was canceled due to poor response. As both ovaries appeared to be devoid of any follicles, she was placed on transdermal estradiol 0.1 mg every 3 days with weekly ultrasound examinations. Her FSH subsequently decreased from 48.5 mIU/mL to 28.7 mIU/mL after 14 days of estradiol treatment. At this point, two tiny follicles were detected on ultrasound and GnRH-ant treatment in the form of daily subcutaneous Ganirelix acetate injection was initiated. This combination of transdermal

estradiol and subcutaneous GnRH-ant resulted in further reduction of FSH levels to 7 mIU/mL. Menses was induced with a 10-day course of oral medroxyprogesterone acetate 10 mg. Baseline hormonal parameters on cycle day 2 of menses were as follows: E2 104 pg/mL; FSH 5.11 mIU/mL; LH 2.76 mIU/mL. Transdermal estradiol was discontinued, and ovarian stimulation was initiated with 150 units of recombinant FSH, 150 units of human menopausal gonadotropin (hMG) and daily Ganirelix acetate. Following 14 days of ovarian stimulation, two dominant follicles developed in the right ovary, and 10,000 IU of human chorionic gonadotropin (hCG) was administered as the ovulatory trigger. Two oocytes were retrieved 36 h later, both of which were mature and fertilized successfully with ICSI. A single day-3 13-cell embryo was transferred, which resulted in a clinical pregnancy. A single day-five, 3 BB blastocyst was also cryopreserved. The patient's non-invasive prenatal screening at 10-week gestation returned normal, and she currently reports an ongoing clinical pregnancy at 16 weeks. The current case highlights that ovarian reserve parameters in isolation may not predict the probability of achieving a pregnancy with IVF, especially in young women with poor ovarian reserve. Critically, when basal LH levels are elevated, it is important to suppress LH levels as soon as stimulation is begun in order to prevent premature luteinization of growing follicles.

Conclusion

Infertility is a common clinical condition, and ART, specifically IVF, remains its quintessential treatment strategy. Efficient ovarian stimulation protocols, standardization oocyte retrieval technique, and successful laboratory techniques have contributed to improved live birth rates and lower complications. Increasing rates of SET cycles have further mitigated perinatal outcomes such as PTB, LBW, and perinatal mortality. Clinical data regarding the long-term health of ART-conceived children remain reassuring.

Suggested Readings

1. Huang JY, Rosenwaks Z. Assisted reproductive techniques. Methods Mol Biol. 2014;1154:171–231.
2. Palermo GD, Kocent J, Monahan D, Neri QV, Rosenwaks Z. Treatment of male infertility. Methods Mol Biol. 2014;1154:385–405.
3. Pereira N, Petrini AC, Hancock KL, Rosenwaks Z. Fresh or frozen embryo transfer in in vitro fertilization: an update. Clin Obstet Gynecol. 2019;62(2):293–9.
4. Sunderam S, Kissin DM, Zhang Y, Jewett A, Boulet SL, Warner L, Kroelinger CD, Barfield WD. Assisted Reproductive Technology Surveillance-United States, 2018. MMWR Surveill Summ. 2022;71(4):1–19.
5. Palermo GD, Neri QV, Schlegel PN, Rosenwaks Z. Intracytoplasmic sperm injection (ICSI) in extreme cases of male infertility. PLoS One. 2014;9(12):e113671.

Chapter 31
Ovarian Hyperstimulation Syndrome (OHSS)

Alexis Melnick and Zev Rosenwaks

Case Part 1

A 33-year-old G0 with a history of polycystic ovary syndrome presents to the emergency room 2 weeks after an intrauterine insemination (IUI) performed by an outside reproductive endocrinologist. We were consulted on her complaint of increasing abdominal distention and discomfort. She reports feeling well until 2 days post-IUI when she began to notice increased abdominal distention and intermittent, sharp pain particularly with ambulation and movement. For the past 2 days, she reports nausea and vomiting and has not been able to keep down both solids and liquids for the past day. She denies fevers, chills, chest pain, shortness of breath or dizziness. She notices that she has been urinating less than usual and reports a weight gain of 8 pounds since the start of her IUI cycle. She provides a summary of her recent treatment from her doctor:

– IUI cycle #1: Clomiphene citrate 50 mg/day (day 3–7), max E2 400 pg/mL, developed 1–2 follicles, neg pregnancy test.
– IUI cycle #2 (current cycle which brought her to the ER):

  FSH 150 iu + hMG 75 iu starting dose on cycle day 2.
  Decreased to 75 iu +75 iu starting cycle day 7 till day of trigger,

A. Melnick · Z. Rosenwaks (✉)
Reproductive Medicine and Ob/Gyn, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: alm2036@med.cornell.edu; zrosenw@med.cornell.edu

5 follicles >/=15 mm on day of trigger (10,000 iu hCG)
E2 1998 pg/mL at trigger.
E2 3116 pg/mL on day of IUI.

Her gynecologic history is significant for PCOS diagnosed in her late teens. She typically has 6–8 menstrual periods per year and is unable to track her ovulation with ovulation predictor kits. Both she and her partner had otherwise normal work-ups with her fertility doctor. Her past medical and surgical history are unremarkable other than an uncomplicated laparoscopic appendectomy at age 14. She takes a daily prenatal vitamin and acetaminophen as needed for occasional headaches.

Evaluation

Evaluation of this patient should include:

- Detailed history of fertility treatment including type and doses of medication, peak estradiol levels, number of follicles at ovulatory trigger, number of eggs retrieved/embryos transferred (if IVF).
- Complete physical exam including:

    - Vitals signs.
    - Weight.
    - Abdominal circumference.
    - Abdominal exam.
    - Lower extremity exam.

- Laboratory evaluation including:

    - Serum hCG.
    - Estradiol (E2), Progesterone (P4).
    - CBC.
    - Basic metabolic profile.
    - Hepatic function profile.

- Imaging:

    - Pelvic sonogram.
    - Chest X-ray (if there is concern for pleural effusion or pulmonary edema).
    - Dopplers (if there is concern for venous thromboembolism).

Differential Diagnosis

- Ovarian hyperstimulation syndrome.
- Ruptured ovarian cyst.

- Ovarian torsion.
- Hemoperitoneum.
- Hyperreactio luteinalis.

Case Part 2

On initial examination, she appears to be comfortable overall. Vital signs are significant for heart rate of 105 bpm, blood pressure of 90/60, and weight of 141 pounds (reports baseline weight of 133–135 pounds). Her lungs are clear to auscultation bilaterally. On abdominal exam, she is noted to have moderate distension with mild tenderness to palpation in the lower quadrants and suprapubic region. A fluid wave is also noted. Lower extremity exam is unremarkable. Laboratory evaluation is significant for a hematocrit of 47%, sodium of 136 mEq/L, and creatinine of 1.1 mg/dL. A quantitative serum hCG is 220 mIU/mL. Pelvic sonogram reveals marked bilateral enlargement of the ovaries with the right and left ovary measuring $10.2 \times 9.1 \times 9.2$ cm and $9.8 \times 6.5 \times 5.4$ cm, respectively, and moderate free fluid in the anterior and posterior cul-de-sacs.

Discussion

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication associated with controlled ovarian hyperstimulation (COH) during assisted reproductive technology (ART) treatment. Though rare (incidence ranges from 0.1 to 5% of all ART cycles depending on treatment type), it a serious and potentially life-threatening condition that requires diligent diagnosis and management. It can occur in all ART clinical settings—oral ovulation induction with clomiphene citrate or aromatase inhibitors (incidence of moderate to severe disease is very low), ovulation induction with exogenous gonadotropins, or in vitro fertilization (IVF). Symptoms include ovarian enlargement, ascites, hemoconcentration, hypercoagulability, and electrolyte abnormalities that are classified by their severity as seen in the table below. In its most severe form, OHSS can lead to serious complications including pleural effusion, acute renal insufficiency, and venous thromboembolism. Symptom presentation is usually described as either early: typically 3–7 days after ovulatory trigger and mediated by exogenous hCG or late: typically at least 9 days after trigger administration and mediated by endogenous hCG due to pregnancy.

The pathophysiology of OHSS involves the sequalae of ovarian enlargement and increased vascular permeability. Increased arteriolar vasodilation and increased capillary permeability leads to third spacing of fluid from intra- to extra-vascular spaces. This fluid shift leads to intravascular hypovolemia in the setting of fluid overload which explains the clinical and laboratory findings seen on initial presentation. Vascular permeability is thought to be mediated by several vasoactive factors secreted by the enlarged ovary, with vascular endothelial growth factor (VEGF) playing the central role. VEGF acts directly on endothelial cells to induce proliferation and angiogenesis and is involved in follicular growth and corpus luteum

function. VEGF is stimulated by hCG (either exogenous or endogenous) and severity of OHSS is positively correlated with both VEGF and hCG levels. In patients who are not pregnant, OHSS typically resolves by the time of the next menstrual period. However, in patients who do conceive, rising hCG levels continue to stimulate the ovaries and its vasoactive mediators and symptoms can persist through the end of the first trimester.

Management of OHSS is largely supportive and therefore, identifying patients at risk for development of the syndrome and preventing its development is crucial. Risk factors include:

- Young age (<35 years old).
- Low BMI.
- High antral follicle count (AFC).
- High basal anti-Mullerian hormone (AMH) level.
- High number of growing follicles/oocytes retrieved.
- Serum E2 levels >2500 pg/mL, >11 follicles on day of trigger (in IVF).
- History of OHSS.
- Polycystic ovary syndrome (PCOS).
- hCG trigger,

Classification of OHSS symptoms

OHSS stage	Clinical feature	Laboratory feature
Mild	Abdominal distension/discomfort	No important alterations
	Mild nausea/vomiting/diarrhea	
	Mild dyspnea	
	Enlarged ovaries	
Moderate	Mild features	Hemoconcentration (Hct >41%)
	Ultrasonographic evidence of ascites	Elevated WBC (>15,000 mL)
Severe	Mild and moderate features	Severe hemoconcentration (Hct >55%)
	Clinical evidence of ascites	WBC >25,000 mL
	Hydrothorax	Creatinine clearance <50 mL/min
	Severe dyspnea	Creatinine>1.6 mg/dL
	Oliguria/anuria	Na + <135 mEq/L
	Intractable nausea/vomiting	K+ >5 mEq/L
		Elevated liver enzymes
	Low blood/central venous pressure	
	Pleural effusion	
	Rapid weight gain (>1 kg in 24 h)	
	Syncope	
	Severe abdominal pain	
	Venous thrombosis	
Critical	Anuria/acute renal failure	Worsening of findings
	Arrhythmia	
	Thromboembolism	

OHSS stage	Clinical feature	Laboratory feature
	Pericardial effusion	
	Massive hydrothorax	
	Arterial thrombosis	
	Adult respiratory distress syndrome	
	Sepsis	

Adapted from the Practice Committee of the American Society for Reproductive Medicine. Prevention and treatment of moderate and severe OHSS. Fertil Steril 2016

Because there is no specific treatment for OHSS, identifying patients at risk is key to lowering its incidence. Prevention strategies should begin prior to treatment with protocols tailored to each patient's individual parameters. Medication dosages should be based on an individual's age, BMI, ovarian reserve, and prior stimulation history, with lower doses used for those patients considered at higher risk for development of symptoms. In IVF, GnRH antagonist protocols are associated with a lower incidence of OHSS as compared to GnRH agonist protocols regardless of trigger type. Incorporating a GnRH antagonist into IVF protocols allows for the use of a GnRH agonist trigger, which has been shown to significantly reduce the incidence of OHSS. Once stimulation has begun, other strategies that can be used to lower the risk of OHSS are step-down protocols (lowering of gonadotropin doses as follicle sizes increase) and coasting (withholding of gonadotropins for up to 4 days). Because hCG is so highly associated with the development of OHSS, withholding hCG and cycle cancelation may be the only option for those patients deemed at very high risk for OHSS.

Choice of trigger for final oocyte maturation is also crucial for prevention of OHSS. The use of hCG for trigger was once standard of care; however, the long half-life of hCG leads to sustained LH-like activity and stimulation of the ovaries post-retrieval, thereby increasing the risk of OHSS. Modifying the dose of hCG to doses lower than the standard 10,000 IU (2500–5000) has been shown to lead to adequate oocyte maturity with lower risk of OHSS. Dose adjustment should be done according to patient estradiol level and BMI. GnRH agonist trigger (2–8 mg Leuprolide acetate) induces endogenous LH release, which has a much shorter half-life than hCG and as such, significantly reduces the incidence of both early and late forms of OHSS when used either alone or in conjunction with a much lower dose of hCG (~1500 IU). Because of LH's shorter half-life, luteolysis occurs early and luteal support with both estradiol and progesterone are required. It is important to remember that a GnRH agonist trigger can only be utilized in GnRH antagonist cycles, as GnRH agonist downregulated patients will not respond to an agonist trigger. Furthermore, even in the setting of antagonist cycles, a small subset of patients (~5%) will fail to elicit an adequate LH response to the GnRH agonist trigger. Identifying these risk factors (history of hypothalamic amenorrhea, LH levels <0.1 at cycle start or day of trigger, long-term OCP use) prior to trigger selection is critically important.

Studies have shown that cabergoline, a dopamine agonist, blocks the increase in vascular permeability via dephosphorylation of VEGF receptors. Therefore administration of cabergoline has been utilized to reduce both the incidence and severity of OHSS in several randomized controlled studies without lowering pregnancy rates. Ideally, cabergoline should be started early, either on the day of trigger or oocyte retrieval, and continued for several days post-procedure (0.5 mg/day for 5–8 days). Those patients with established OHSS may also benefit from treatment with cabergoline in order to lessen symptom severity and duration. For patients undergoing IVF who are at risk for OHSS, embryo cryopreservation should always be considered. Given the efficiency of embryo vitrification, high survival rates post-thaw, and equivalent or even superior pregnancy rates with frozen as compared to fresh embryo transfers, foregoing a fresh transfer and returning for subsequent frozen transfer is an effective way to reduce the risk of late onset OHSS. Lastly, luteal phase support with progesterone, rather than hCG, should always be used in patients at risk for development of OHSS.

Management of OHSS should be directed at alleviating symptoms and stabilizing the hemodynamic aberrations. Most cases will resolve spontaneously over time although pregnant women may have symptoms throughout the entire first trimester and should be watched more carefully for signs of illness progression. Most patients with mild or moderate disease can be managed on an outpatient basis with oral analgesics, anti-emetics, pelvic rest, and frequent visits to monitor vital signs including weights, ultrasound examinations to assess the degree of ascites and/or ovarian enlargement, and serial laboratory testing of electrolytes, creatinine, CBC, and liver function. Given the relative state of intravascular hypovolemia, hydration is key, and patients should be directed to drink no less than 1 L of fluid per day. Prophylactic anticoagulation (low molecular weight heparin 40 mg daily or subcutaneous heparin 5000 units BID) should be considered in women with 2–3 of the following risk factors in addition to OHSS: age > 35, obesity, immobility, elevated hematocrit, pregnancy, thrombophilias, and history of venous thromboembolism. As resolution of increased vascular permeability ensues, reentry of third spaced fluid into the intravascular space and natural diuresis will occur, improving hemoconcentration and ascites [1–7].

Hospitalization is necessary for patients with severe disease and those with any of the following symptoms:

- Intractable nausea/vomiting preventing ingestion of adequate food/fluids.
- Severe abdominal pain or peritoneal signs.
- Tachycardia.
- Hypotension, dizziness, or syncope.
- Dyspnea.
- Tachypnea.
- Tense ascites.
- Severe hemoconcentration (Hct >45%).

- Electrolyte abnormalities (Na <125 mEq/L, K >5 mEq/L).
- Decreased creatinine clearance (Cr >1.2 mg/dL).
- Elevated liver enzymes.

Hospitalized patients require frequent monitoring of vital signs, body weights and abdominal circumferences, intake and output (I&Os), serial lab evaluations, and imaging as indicated (i.e., chest X-ray if shortness of breath). Fluid management is critical and normal saline is preferred over lactated ringers given the often-observed hyponatremia/hyperkalemia. For those patients with intractable nausea/vomiting and/or pain, adequate doses of analgesics and/or anti-emetics should be provided. Pregnant women should also receive any necessary luteal support with progesterone. All hospitalized patients should receive prophylactic anticoagulation and wear lower extremity sequential compression devices while in bed. Ultrasound-guided paracentesis may be indicated for patients with ascites that causes pain, compromised pulmonary function, or oliguria/anuria that does not improve with appropriate fluid management. Both transvaginal and transabdominal approaches can be used depending on provider preference. While the absolute volume of fluid to be removed via paracentesis is not well established, fluid should be removed slowly, while carefully monitoring patient's response. Volume expanders such as albumin are often given in conjunction with paracentesis although the data regarding their efficacy is limited. Serial paracentesis may be required to maintain adequate renal and pulmonary function. Management in an intensive care unit maybe necessary for patients with thromboembolic complications, renal failure, or pulmonary compromise not responsive to supportive care and paracentesis.

Case Part 3

The patient was admitted to the hospital for management of her OHSS. She was initially given a bolus of 1 L of normal saline and then maintained at 125 cc/h. A foley catheter was placed to monitor urine output. She was also started on 5000 iu of subcutaneous heparin twice daily for DVT prophylaxis. After being started on anti-emetics, she was able to tolerate small amounts of food/drink orally. After some initial symptom relief and slight improvement in labs and urine output, she was noted to have worsening abdominal distension and discomfort on hospital day 3. Labs showed improvement in hemoconcentration (Hct 43%) but an increase in her creatinine (1.4). Urine output has also decreased to <20 cc/h. Given her deteriorating renal function and discomfort, she underwent an ultrasound-guided transabdominal paracentesis performed at interventional radiology. A total of 1.5 L of ascitic fluid was removed and renal function improved dramatically over the next 24 h. By hospital day 5, she reported significant improvement in symptoms—she was only mildly distended and was tolerating a regular diet. Electrolytes and creatinine were all within normal range and hematocrit was stable at 44%. Her hCG level was monitored and continued to rise appropriately throughout her stay. She was discharged to home on hospital day 7 with a plan for close outpatient follow-up by her local reproductive endocrinologist.

References

1. Busso CE, Reis Soares S, Pellicer A. In: Martin KA, editor. Management of ovarian hyperstimulation syndrome. Waltham, MA: UpToDate; 2021a. Accessed 1 May 2022.
2. Busso CE, Reis Soares S, Pellicer A. In: Martin KA, editor. Pathogenesis, clinical manifestations, and diagnosis of ovarian hyperstimulation syndrome. Waltham, MA: UpToDate; 2021b. Accessed 1 May 2022.
3. Chen D, Burmeister L, Goldschlag D, Rosenwaks Z. Ovarian hyperstimulation syndrome: strategies for prevention. Reprod Biomed Online. 2003;7(1):43–9.
4. Kashyap S, Parker K, Cedars MI, Rosenwaks Z. Ovarian hyperstimulation syndrome prevention strategies: reducing the human chorionic gonadotropin trigger dose. Semin Reprod Med. 2010;28(6):475–85.
5. Navot D, Bergh P, Laufer N. The ovarian hyperstimulation syndrome. In: Adashi EY, Rock JA, Rosenwaks Z, editors. Reproductive endocrinology, surgery and technology. Philadelphia, PA: Lippincott-Raven; 1996. p. 2215–32.
6. Practice committee of the American Society for Reproductive Medicine. Ovarian hyperstimulation syndrome. Fertil Steril. 2008;9(5 Suppl):S188–93.
7. Practice committee of the American Society for Reproductive Medicine. Prevention and treatment of moderate and severe ovarian hyperstimulation syndrome: a guideline. Fertil Steril. 2016;106(7):1634–47.

Chapter 32
Pre-Implantation Genetic Testing

Glenn Schattman

Case

SR, a 35-year-old G1P0010 cisgender female, initially presented with her husband for secondary infertility. She discontinued oral contraceptive pills approximately 2 years ago and immediately conceived a pregnancy in the first cycle they tried but this pregnancy ended in an early miscarriage. In a subsequent cycle when she was late for her period again by about 1 week, she performed a urine pregnancy test at home which was positive. Initial BhCG at her physician's office rose to a maximum level of 682 mIU/mL within 1 week, followed shortly by a decline culminating in a menstrual period. Transvaginal ultrasound was never performed since the BhCG never rose above the threshold of 1500 mIU/mL needed to visualize an intrauterine gestational sac. After the miscarriage, the couple tried to conceive again for 9 months without success. They were referred to a reproductive endocrinologist whose workup included a hysterosalpingogram which showed a normal uterine cavity with bilateral tubal patency, a normal semen analysis and compatible genetic testing revealing that they were not at risk of having a child affected by a devastating genetic disease tested on the panel. Pelvic ultrasound demonstrated a normal uterus and no adnexal mass. Antral follicle count was 14, the expected mean for her chronologic age. Additional measures of ovarian reserve obtained revealed an anti-Mullerian hormone (AMH) level of 1.4 ng/mL, consistent with the antral follicle count. The couple stated that ideally, they would like to have two children.

They were counseled about the results of all the testing and options for improving the odds of conceiving a successful pregnancy. They were treated with clomiphene citrate (50 mg/day × 5 days) with appropriately timed intrauterine insemination

G. Schattman (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: glschatt@med.cornell.edu

231

(IUI) for 3 months. She had a good response to the clomiphene citrate with 2 or 3 follicles developing each time but unfortunately, no pregnancy resulted. Options were reviewed with the couple including continuing clomiphene citrate with IUI for up to 2–3 more cycles or proceeding with in vitro fertilization (IVF). During the consultation, the couple raised the question about genetically testing their embryos for aneuploidy. They mentioned that they have many friends who underwent IVF and were told to proceed with genetic testing of their embryos as it would increase the probability of pregnancy while reducing the chance of miscarriage.

Discussion

Is IVF the next appropriate step for this couple? The simple answer to that question would be "yes." This couple is experiencing secondary infertility and the most likely cause of the pregnancy loss of the first pregnancy statistically would be embryo aneuploidy. Since the workup has been unrevealing, and medicated IUI has not worked, IVF is an appropriate next step. As the majority of pregnancies with controlled ovarian stimulation and IUI occur within the first three attempts, continuing with IUI treatment is less likely to be successful.

IVF involves stimulation with gonadotropins to induce multi-follicular development with the retrieval of mature metaphase II (MII) oocytes. The number of oocytes that can be retrieved is directly correlated to the individual's ovarian reserve. In this patient with an AMH of 1.4 ng/mL and an AFC of 14, one would anticipate that the average number of oocytes available each month would be ~14 ± 2. Assuming that the majority of oocytes (80%) will be mature and that 70% fertilize, this will result in approximately eight fertilized (2PN) zygotes each cycle. Now, assuming that between 25 and 50% of 2PN zygotes survive extended in vitro culture to blastocyst stage, there would be between 2 and 4 blastocysts from which to select for transfer on day 5. In fact, in one study [1], the mean # of blastocysts available in a 35-year-old woman was 4. The mean # blastocysts was in younger patients was 6, decreasing to a mean of 2 at age 40.

Generally, there is agreement that IVF is the most efficient step forward for this couple in order to achieve a successful pregnancy. We should also agree that the reason why older women have a lower probability of implantation/pregnancy along with a higher miscarriage rate is because each egg they ovulate and embryos they create has an increased probability of being chromosomally abnormal. Why should we then not recommend to genetically test every embryo for aneuploidy before transfer when performing IVF?

Taking a closer look at pre-implantation genetic testing (PGT) of embryos, there are many reasons to genetically test an embryo, PGT-M, PGT-A, and PGT-SR. PGT-M is the acronym used for "M"onogenic diseases which can be autosomal recessive, dominant or sex linked. For recessive disorders like cystic fibrosis, the risk of each embryo being affected is roughly 25% (50% will be unaffected carriers) and for dominant disorders each embryo has a 50% of being affected (consider BRCA or

Lynch Syndrome). PGT-SR evaluates for "S"tructural "R"earrangements such as unbalanced karyotypes in embryos from couples where one of the parents has a balanced translocation. In both of these scenarios (M and SR), the indications for PGT are for disease avoidance and reducing miscarriage due to a genetically inherited aberration.

PGT-A however is evaluating the embryo for a de-novo "A"neuploidy arising from either a meiotic error or mitotic error post-fertilization. This testing is different from PGT-M and PGT-SR in that parents are normal (euploid) and not directly transmitting a mutated gene to the embryo. Meiotic errors in either the oocyte or sperm will lead to aneuploidy resulting in the same abnormality in every cell of the embryo. Mitotic errors post-fertilization will lead to mosaic embryos where not every cell will be affected depending on the developmental stage of the embryo when the error occurred. This phenomenon of mosaicism leads to potential diagnostic errors when analyzing a few cells (out of ~120 cells) from an embryo 5–6 days post-fertilization. Since trophectoderm biopsies taken at random do not represent the inner cell mass, and cells divide in a clonal fashion, a single biopsy of a few cells may not represent the genetic health of the entire embryo.

Careful evaluation of the studies describing the benefits of PGT-A suggest that they were biased by flaws in study design including improper primary outcomes, highly selected good prognosis populations, inappropriate randomization by including patients only when they had achieved multiple good quality blastocysts for analysis. These design flaws make the studies biased toward benefitting only in a small and highly selected population and may not represent the majority of IVF patients. PGT-A proponents have used these studies for years to mass-market PGT-A to all patients undergoing IVF.

The largest and most relevant study looking at real world patient populations is a recent multi-center, international study called the "STAR trial" [2]. In this study, 984 patients up to 40 years of age were randomized to either PGT-A group or a control group. The PGT-A group had all of the blastocysts biopsied on day 5 or 6 of development and cryopreserved immediately after biopsy. Embryo transfer was done in the subsequent cycle selecting the best euploid embryo for transfer. The control group had the best quality blastocyst cryopreserved on D5 while all of the other embryos biopsied and cryopreserved on D5 or D6. All patients then underwent transfer of either the best, euploid embryo in the PGT-A group or the single best, non-biopsied embryo that was chosen on D5 in the control group. Outcomes were then analyzed by implantation rate per transfer.

Only 661 patients (330 PGT-A, 331 control) out of the original 984 randomized were included in the final analysis. Despite selecting for only good prognosis patients based on AMH and AFC, 70/510 (13.7%) patients aged <35 and 97/474 (20.5%) aged 35–40 did not have at least two good quality blastocysts for analysis and were excluded. Of the remaining 330 patients in the PGT-A group, only 274 (83%) could be included because 67 patients (20.5%) had no normal embryos available for transfer and in six patients (1.8%) the embryo did not survive the thaw. While the implantation rate overall was "higher" for the PGT-A group, when you take into account the patients who did not have a transfer, the ongoing pregnancy

rate (>20 weeks gestation) was 41.5% in the PGT-A group and 45.6% in the control group. Since the patient population covered a wide range of ages, a careful analysis comparing younger patients (aged <35) and older patients (aged 35–40) is warranted. Younger patients were potentially negatively impacted by testing their embryos. Implantation rate (+BHCG/embryo transferred) of highly selected embryos was not different between the two groups either (49.3% and 53% for PGT-A and control, respectively). The ongoing pregnancy rate was lower in the PGT-A group (42.5%) compared to the control group (50.3%).

In the older group (aged 35–40), where aneuploidy rates are higher, the implantation rate was noted to be higher for PGT-A tested embryos (50.8%) than in the control group (37.2%). But when evaluated by intention to treat (ITT) including the patients who did not have an embryo suitable for transfer, ongoing pregnancy rates were not statistically different between the two groups, 41.1% and 35.7% for the PGT-A and control groups, respectively. Additionally, miscarriage rates were also not different between the two groups, even in the older population with 8.2% miscarriage rate for tested embryos and 11% for untested embryos. Interestingly, as discussed previously, random biopsies reveal mitotic errors with the sample of cells being "mosaic" mixes of normal and abnormal cells. This mosaic finding was present in about 16.5% of all embryos regardless of patient age. When we take into account the number of mosaic but potentially viable embryos discarded with PGT-A, the cumulative pregnancy rate per stimulated cycle may be even lower in the PGT-A group.

In a recently published trial in the NEJM [3], cumulative live birth rates per stimulation cycle were evaluated using PGT-A compared to controls. Patients ages 20–37 were randomized when they had at least three blastocysts. There were 606 patients randomized to the PGT-A group and 606 randomized to the control group for 1212 patients in total. Cumulative live birth rates within the first year were analyzed until no embryos remained within the first year. In the PGT-A group, the total number of live births was 468 (77.2%) per started cycles. In the control group, this rate was actually higher at 81.8% (496 live births)! In fact, even the miscarriage rate was not significantly different (12.6% for untested embryos vs. 8.7% in the tested embryos).

In conclusion, PGT is the process of removing one or more cells from a developing embryo for genetic testing. PGT can be performed for patients at risk of having children with a serious monogenic condition, recurrent pregnancy loss due to one of the parents carrying a balanced translocation or a de-novo aneuploidy. Disease avoidance and reducing the probability of miscarriage can be done with the primary outcome being the birth of a healthy child without disease. PGT-A on the other hand has been promoted as a means to improve live birth rates, decrease the time to pregnancy and reduce miscarriage rates. As can be seen from the data presented herein, PGT-A does not deliver on any of these indications. While implantation rates per embryo transferred were higher in women >35, this improvement comes at the expense of a lower take home baby rate, especially if you consider that the patient most likely to have only 1 or 2 embryos available per stimulation cycle is the patient over 35.

So who may benefit from PGT-A? Clearly PGT-A should not be routinely offered for patients up to aged 35. Exceptions may apply to patients desiring to choose the gender of their children. In all other situations, we can offer transfer of the best and untested embryo, and test and cryopreserve the remaining blastocysts. In patients aged 35 and older, PGT-A should only be offered in patients who have an excess number of embryos (i.e., >4) and they should consider a fresh embryo transfer if it is safe to do so with biopsy performed on the remaining embryos.

PGT-A does not appear to reduce the miscarriage rate per embryo transferred, even in older patients as most abnormal embryos will either not implant or result in a pre-clinical loss. PGT-A testing of all embryos does not reduce the time to pregnancy since the delay of 1 month to biopsy and wait for the genetic results imparts a delay to pregnancy not imposed on the patient proceeding with a fresh transfer. To date, PGT-A, without a demonstration of an improvement in live birth rates, is not cost-effective. In fact, PGT-A appears to be more expensive since it leads to a lower cumulative pregnancy rate, requiring more cycles of ART to achieve a successful pregnancy.

References

1. Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, et al. The nature of aneuploidy with increasing age of female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil Steril. 2014;101:656–63.
2. Munne S, On behalf of the STAR Study Group. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. Fertil Steril. 2019;112:1071–9.
3. Yan J, Qin Y, Zhao H, Sun Y, Gong F, Li R, et al. Live birth with or without pre-implantation genetic testing for aneuploidy. N Engl J Med. 2021;385:2047–58.

Chapter 33
Planned Fertility Preservation

Nina Vyas and Dan Goldschlag

Case

A 32-year-old female patient and her 30-year-old partner of 2 years presented to our office requesting to review their fertility preservation options.

The couple expressed their wish of starting a family with two children in the next 6–7 years. At this time, the patient could not consider conception any sooner. They also conveyed their discomfort with the creation and storage of embryos. Both partners requested a fertility assessment and wished to further understand their planned fertility preservation options.

The patient underwent a pelvic ultrasound and hormonal testing. She was found to have a normal appearing uterus and 10–12 antral follicles within each ovary. Her anti-Mullerian hormone (AMH) level was 6.62 ng/mL. Her partner underwent a semen analysis which had grossly normal parameters.

Couple was counseled extensively about various fertility preservation options including cryopreservation of embryos (EC), oocytes (OC), sperm, and ovarian tissue (OTC). Partners both agreed that oocyte cryopreservation would best meet their needs.

She was started on an estrogen priming protocol followed by 75 IU of follicle stimulating hormone (FSH) and 150 IU of human menopausal gonadotropins (HMG) administered daily. Gonadotropin releasing hormone antagonist was begun on day 6 of ovarian stimulation. Human chorionic gonadotropin was given after 9 days of ovarian stimulation once they were two follicles measuring 18 mm. Her estradiol at time of trigger was to 2279 pg/mL. Nineteen oocytes were retrieved, of which 17 were mature and frozen. Patient was counseled on her potential success

N. Vyas · D. Goldschlag (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: degoldsc@med.cornell.edu

rates of achieving a pregnancy in the future given the number of oocytes cryopreserved. The couple was encouraged to return for follow-up in 1–2 years to further discuss their procreative plans.

Discussion

There are many medical indications for fertility preservation such as imminent gonadotoxic therapies, genetic conditions, fertility preservation prior to gender-affirming therapies, and others that are discussed in detail in a separate chapter. Planned oocyte cryopreservation (OC) or embryo cryopreservation (EC), which refers to the process of electively choosing to cryopreserve mature oocytes or embryos, has become popular with increasing numbers of young women desiring to maintain fertility for future family building while guarding against the age-related decline in the naturally observed fecundity.

Historically, slow-freeze techniques were utilized for cryopreservation of oocytes in an effort to minimize intracellular ice formation. The first birth from a frozen oocyte utilizing this methodology was reported in 1986 [1]. However, given the large physical size of oocytes and their water content, they are particularly susceptible to damage from intracellular ice formation. As a result, the spindle apparatus can be damaged thereby disrupting the chromosome alignment at metaphase-II. Considering this limitation, survival rates after thaw, fertilization, and embryo development were poor when oocytes were frozen by the slow-freeze method.

More recently vitrification, an ultrarapid cooling or flash-freezing technique that solidifies the oocyte into a glass-like state and prevents intracellular ice formation, we have witnessed dramatically improved oocyte survival rates [2]. Since the first report of a baby born using this technology in 1999 [3], vitrification has become the method of choice for oocyte cryopreservation.

Moreover, this novel technique has led to an enormous expansion in the use of OC due to its improved efficiency and outstanding pregnancy rates. However, it should be emphasized that patients should be counseled regarding the ideal age for fertility preservation and the optimal number of oocytes needed to achieve a live birth. The first study providing clinical data on live births after planned OC using vitrified eggs was reported in 2013 by Cobo et al. [4] and an update by the same group was published in 2016 [5]. Live birth rates per patient was 50% in women ≤35 years vs. 22.9% in those >35 years. The same study showed that success rates decreased significantly in patients >40 years (3.7%). The authors estimated that 8–10 mature oocytes are required to achieve a reasonable success in women <35 years. Another group analyzed 128 autologous IVF cycles using previously cryopreserved oocytes to estimate the number of eggs needed for a 70% chance of one live birth [6]. They found that a women aged 30–34 would need to cryopreserve 14 mature oocytes, women aged 35–37 would need 15 mature oocytes, and women aged 38–40 would need 26 mature oocytes. Although these numbers serve as a useful estimate, clinicians are responsible for counseling women that there is no

definitive data on the "target number" that guarantees a pregnancy at any age after oocyte freezing.

Of interest, women with endometriosis (especially symptomatic or severe) have poorer outcomes in fertility preservation [7, 8]. Surgical excision of the endometrioma before the collection of oocytes negatively affected the outcome of OC. Therefore, egg freezing should be encouraged before surgery is performed in these women.

Despite the increased popularity of planned OC, most recent studies [9–11] have demonstrated that the return rate to utilize the cryopreserved oocytes is only between 12% and 38% in women who were followed up to 10–15 years. While the mean age of first cryopreservation has been steadily declining in recent years, most of the reporting patients who came back to use their frozen eggs appeared to be in the older age groups. When couples are choosing between EC and OC, as in our case presentation, it is important for them to recognize that cryopreserved embryos, as opposed to eggs, will be under their joint responsibility. Therefore, their use and disposition will have to be jointly agreed upon.

Patients also have to know that cohort studies [12, 13] addressing the obstetric and neonatal outcomes by comparing health concerns between pregnancies from vitrified oocytes vs. fresh embryo transfers did not observe any difference. The autologous cryopreserved oocytes seem to have comparable neonatal outcomes as their fresh counterparts.

In summary, planned OC has become an ever-increasingly sought-after option for women who plan to delay childbearing. However, they need to understand that oocyte freezing cannot guarantee future motherhood but is a reasonable means to improve their chances of having a biological child later in life. Most reviews to date show a significantly higher efficiency in OC in younger women [14–16]. Therefore, patients considering such treatment should be counseled that they should proceed sooner than later. However, providers have to ensure that patients are properly advised on both their realistic pregnancy expectations and the likelihood of their eggs being utilized in the future.

References

1. Chen C. Pregnancy after human oocyte cryopreservation. Lancet. 1986;327(8486):884–6.
2. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the cryotop method. Theriogenology. 2007;67(1):73–80.
3. Kuleshova L, Gianaroli L, Magli C, Ferraretti A, Trounson A. Birth following vitrification of a smaller number of human oocytes: case report. Hum Reprod. 1999;14(12):3077–9.
4. Garcia-Velasco JA, Domingo J, Cobo A, Martinez M, Carmona L, Pellicer A. Five years' experience using oocyte vitrification to preserve fertility for medical and nonmedical indications. Fertil Steril. 2013;99(7):1994–9.
5. Cobo A, Garcia-Velasco JA, Coello A, Domingo J, Pellicer A, Remohi J. Oocyte vitrification as an efficient option for elective fertility preservation. Fertil Steril. 2016;105(3):755–64.
6. Doyle JO, Richter KS, Lim J, Stillman RJ, Graham JR, Tucker MJ. Successful elective and medically indicated oocyte vitrification and warming for autologous in vitro fertilization, with

predicted birth probabilities for fertility preservation according to number of cryopreserved oocytes and age at retrieval. Fertil Steril. 2016;105(2):459–66.

7. Elizur SE, Chian RC, Holzer HE, Gidoni Y, Tulandi T, Tan SL. Cryopreservation of oocytes in a young women with severe and symptomatic endometriosis: a new indication for fertility preservation. Fertil Steril. 2009;91(1):293.

8. Somigliana E, Vercellini P. Fertility preservation in women with endometriosis: speculations are finally over. The time for real data is initiated. Fertil Steril. 2020;113(4):765–6.

9. Cobo A, Garcis-Velasco J, Domingo J, Pellicer A, Remohi J. Elective and onco-fertility preservation: factors related to IVF outcomes. Hum Reprod. 2018;33(12):2222–31.

10. Fuchs Weizman N, Baram S, Montbriand J, Librach CL. Planned oocyte cryopreservation (planned OC): systematic review and meta-analysis of cost-efficiency and patients' perspectives. BJOG. 2021;128(6):950–62.

11. Leung AQ, Baker K, Vaughan D, Shah JS, Korkidakis A, Ryley DA, et al. Clinical outcomes and utilization from over a decade of planned oocyte cryopreservation. Reprod Biomed Online. 2021;43(4):671–9.

12. Cobo A, Serra V, Garrido N, Olmo I, Pellicer A, Remohi J. Obstetric and perinatal outcome of babies born from vitrified oocytes. Fertil Steril. 2014;102(4):1006–15.

13. Anzola AB, Pauly V, Geoffroy-Siraudin C, Gervoise-Boyer MJ, Montjean D, Boyer P. The first 50 live births after autologous oocyte vitrification in France. J Assist Reprod Genet. 2015;32(12):1781–7.

14. Cobo A, Garcia-Velasco JA, Remohi J, Pellicer A. Oocyte vitrification for fertility preservation for both medical and nonmedical reasons. Fertil Steril. 2021;115(5):1091–101.

15. Walker Z, Lanes A, Ginsburg E. Oocyte cryopreservation review: outcomes of medical oocyte cryopreservation and planned oocyte cryopreservation. Reprod Biol Endocrinol. 2022;20(1):10.

16. Daar J, Benward J, Collins L, Davis J, Davis O, Francis L, et al. Planned oocyte cryopreservation for women seeking to preserve future reproductive potential: an ethics committee opinion. Fertil Steril. 2018;110(6):1022–8.

Chapter 34
Fertility Preservation: Medical

Dan Goldschlag

Case

A 13-year-old female patient and her parents present to our office requesting to discuss her fertility preservation options. Two days earlier she had seen her pediatrician secondary to a 3-day history of nausea, vomiting, and episodes of syncope. Significant laboratory findings included a complete blood count (CBC) with pancytopenia prompting a referral to hematology/oncology.

A bone marrow biopsy was performed with the following results:

– 1% blasts
– inverted myeloid to erythroid ratio,
– dysplastic erythroid cells.

She was diagnosed with myelodysplastic syndrome and was scheduled to be treated immediately with chemotherapy including busulfan, melphalan, and fludarabine prior to a stem cell transplantation. The patient and her family had been informed that she would lose all ovarian functions posttransplantation and they were referred to our group to discuss the ovarian tissue cryopreservation (OTC) process. The patient and her parents wished to further discuss any other options for preserving her fertility.

Our evaluation demonstrated a well-nourished, 13-year-old girl with a weight of 50 kg and a height of 149 cm. She had Tanner stage 3 breast development and a Tanner stage 1 pubic hair pattern. Patient was premenarcheal by her history. A bimanual pelvic examination revealed a partially intact hymeneal ring. A transabdominal pelvic sonogram demonstrated a small uterus. Ovaries had 4–5 antral follicles bilaterally. Her endocrine labs were as follows:

D. Goldschlag (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: degoldsc@med.cornell.edu

- Estradiol level of 65 pg/mL,
- Follicle-stimulating hormone (FSH) level of 5.0 mIU/mL,
- Luteinizing hormone (LH) level of 2.9 mIU/mL,
- Anti-Mullerian hormone (AMH) level of 0.95 ng/mL.

We reviewed both the process of ovarian tissue cryopreservation (OTC) and oocyte cryopreservation (OC) along with the data regarding future pregnancy rates. Subsequent to counseling, they elected to attempt oocyte cryopreservation. Her oncologists felt that the time required for ovarian stimulation and transvaginal follicular aspiration was medically safe and would not impact upon her treatment success. The patient and her family were also set up for psychological counseling.

She was started on daily 225 IU of human menopausal gonadotropin (HMG). Daily gonadotropin-releasing hormone (GnRHa) antagonist began on day seven of ovarian stimulation. Follicular growth was assessed via transabdominal sonography and serial estradiol measurements. Gonadotropin stimulation was well tolerated by the patient. Recombinant human chorionic gonadotropin was given after 9 days of ovarian stimulation when there were two lead follicles of 18 mm. Her estradiol was 1132 pg/mL on the day of trigger.

One day prior to oocyte aspiration, she was admitted to the hospital to make sure she was hemodynamically stable for the procedure. Her complete blood count (CBC) demonstrated a hematocrit of 19% and platelet count of 15,000/mL. She was transfused with packed red cells (PRBCs) and platelets preoperatively. On the morning of oocyte aspiration, she was given an additional platelet transfusion, resulting in a preoperative platelet count of 120,000/mL.

We obtained 20 oocytes, of which 18 were mature and frozen. The postoperative examination revealed excellent hemostasis, and the patient was transferred to the recovery room. Postop CBC was stable. She was discharged postop day 1, with a hematocrit of 29.8%, and platelet count of 109,000/mL.

Discussion

Historically, it had been assumed that prepubertal patients would not respond to conventional ovarian stimulation, thus unlike adults, pediatric patients facing gonadotoxic therapy are routinely offered ovarian tissue cryopreservation (OTC). This case describes the first ovarian stimulation and oocyte aspiration in a pediatric premenarchal patient who had not completed puberty. Though the limited numbers of live births from ovarian tissue transplantation (OTT) worldwide continue to rise, oocyte cryopreservation remains a less invasive and more efficacious treatment modality. When the time interval between diagnosis and initiating gonadotoxic treatments is limited (<2 weeks), OTC can provide an expedient alternative.

Reproductive capabilities are influenced by factors such as age, ovarian reserve, gonadotoxic medications, radiotherapy, medical conditions, surgical procedures, and genetics factors (POI—Table 34.1). For affected patients, providing

Table 34.1 Non-oncological causes of primary ovarian insufficiency (POI)

Surgical/Medical	Genetic	Autoimmune	Hematologic
Benign ovarian tumors	Galactosaemia	Systemic lupus erythematosus (SLE)	Aplastic anemia
Endometriosis	Fragile X premutations (FMR 1)	Behcet's disease	Beta-thalassaemia
Infectious conditions of the ovaries	Turner's syndrome	Churg-Strauss syndrome	Sickle cell anemia
		Glomerulonephritis	
		Wegener's granulomatosis)	
		Inflammatory bowel diseases	

opportunities for fertility preservation (FP) can be critical for both pre- and postpubertal individuals to complete their future reproductive desires. Identifying the patient population at risk of premature ovarian insufficiency (POI) and referring patients early on in the process is crucial in facilitating effective care.

Depending on the specific diagnosis, individuals at risk of POI should be offered fertility preservation (FP) options such as embryo cryopreservation (EC), oocyte cryopreservation (OC), ovarian transposition (OT), ovarian tissue cryopreservation (OTC) or GnRHa administration. For example, conditions such as Turner's Mosaics and Fragile X premutation carriers can be progressive and may eventually progress to complete ovarian failure. Similarly, surgical interventions for conditions such as endometriosis and oophoritis can lead to POI. As time lapses, the ovarian reserve continues to diminish for these individuals thereby further worsening the POI. Intervening with a preemptive FP strategy early is prudent.

Benign conditions such as autoimmune diseases like lupus and others may be treated with therapeutic regiments including alkylating agents (e.g., Cytoxan). These therapies can lead to POI secondary to their gonadotoxicity. Similarly, some non-oncological hematologic diseases are often best treated by autologous or allogeneic stem cell transplantation (SCT). Patient will be at a very high risk of POI secondary to the chemotherapies and radiotherapy that are utilized to ablate the existing bone marrow. These individuals, if medically stable, should also be offered FP options prior to gonadotoxic treatment.

Finally, oncologic diseases represent the largest group of individuals seeking FP for medical indications. These patients' options can be further limited by the reduced time they have been permitted by their oncologist for FP treatment and the medical complexities of their disease. Cancer treatments using gonadotoxic chemotherapy and/or radiotherapy to the pelvis will diminish or eradicate the ovarian reserve. Ovarian transposition (OT) can be offered when pelvic irradiation is indicated and gonadotoxic medications are not being used. Radiation scatter and the inability to completely shield the ovaries can limit which patients may be appropriate candidates for OT.

For GnRHa, there is limited and inconsistent evidence demonstrating fertility benefits from its effect on ovarian suppression in the presence of gonadotoxic

therapies. Understanding the type of chemotherapies(s) used and their dosages can help estimate the degree of diminished ovarian reserve and risk of POI.

While embryo cryopreservation has long been the gold standard for FP, improvements with vitrification protocols have allowed oocyte cryopreservation to become an effective, viable alternative. Oocyte cryopreservation can be offered to single patients without a committed partner, lesbian couples, and those who are uncomfortable with embryo cryopreservation. For individuals for whom ovarian stimulation is contraindicated, ovarian tissue cryopreservation (OTC) offers the possibility of autologous re-transplantation of her ovarian tissue or of potential future in vitro maturation and fertilization of oocytes. Currently the number of live births worldwide postovarian tissue transplantation is less than a few hundred. OTC remains a less effective (21–23% live birth rate at age <35 live birth rate vs. 47% live birth rate with EC) but proven option for future pregnancies for patients who are unable to participate in OC or EC. OTC does not require an individual to be able to make mature oocytes nor does it require the usual 14 days needed to perform an ovarian stimulation/oocyte retrieval cycle.

In summary, fertility preservation strategies include oocyte cryopreservation, embryo cryopreservation, ovarian transposition, ovarian tissue cryopreservation, and GnRHa administration. Discussing FP options early on with both pre- and postmenarcheal women soon after they learn of their medical conditions will give them the greatest opportunities to evaluate their options and choose their best FP strategy. A team of fertility care practitioners including specialized care coordinators, nurses, physicians, psychologists, and financial coordinators are needed to help patients optimize their care.

Suggested Readings

1. Reichman DE, Davis OK, Zaninovic N, Rosenwaks Z, Goldschlag DE. Fertility preservation using controlled ovarian hyperstimulation and oocyte cryopreservation in a premenarcheal female with myelodysplastic syndrome. Fertil Steril. 2012;98(5):1225–8.
2. Practice Committee of the American Society for Reproductive Medicine. Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. Fertil Steril. 2019;112(6):1022–33.
3. American Society of Clinical Oncology. 2018. www.asco.org/survivorship-guidelines.
4. ESHRE Guideline Group on female fertility preservation, Anderson RA, Amant F, Braat D, D'Angelo A, de Sousa C, Lopes S, Demeestere I, et al. ESHRE guideline: female fertility preservation. Human Reprod Open. 2020;2020(4):hoaa052.
5. Mulder RL, Font-Gonzalez A, Hudson MH, van Santen HM, Loeffen EAH, Burns KC, et al. Fertility preservation for female patients with childhood, adolescent, and young adult cancer: recommendations from the PanCareLIFE consortium and the international late effects of childhood cancer guideline harmonization group. Lancet Oncol. 2021;22(2):e45–56.
6. Oktay K, Harvey BE, Partridge AH, Quinn GP, Reinecke J, Taylor HS, et al. Fertility preservation in patients with cancer: ASCO clinical practice guideline update. J Clin Oncol. 2018;36(19):1994–2000.

Chapter 35
Menopause

Alexis Melnick

Case

A 56-year-old G2P2002 presents with a 3-year history of hot flashes and night sweats. She experiences 10–15 hot flashes each day and wakes up multiple times each night covered in sweat. These symptoms have been persistent, but stable over the last few years. Upon questioning, she states that these symptoms often interfere with her ability to perform well at work. She works as a lawyer in a busy law firm and the daily hot flashes can be unbearable when she is dressed in a suit in court. Additionally, the constant sleep interruptions often lead to her feeling sleepy throughout the day, which affects her ability to focus. She states her mood is relatively stable, though she has noticed increased irritability in the last couple years. Her symptoms are exacerbated by sweating. She used to exercise 4–5 times per week, but this has decreased to once per week due to her heat intolerance. She has gained 10 pounds in the last year.

Her last menstrual period was at age 53. Menarche was at age 12. She had regular monthly menstrual cycles throughout her 20s and 30s without dysmenorrhea. In her late 40s, she notes her menstrual cycles became shorter and more irregular. From age 50–53, intervals between her menstrual cycles then became more prolonged, lasting 60–90 days. She denies any vaginal bleeding since age 53. She reports two pregnancies at age 28 and age 31, both which resulted in uncomplicated term vaginal deliveries.

She is currently in a monogamous relationship with her husband. They have intercourse at least once per week. She denies any pain with intercourse or vaginal dryness. She is up to date on her routine health care maintenance. Her last mammogram 6 months ago was normal. Her past medical history is significant for

A. Melnick (✉)
Reproductive Medicine and Ob/Gyn, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: alm2036@med.cornell.edu

hypothyroidism and asthma. She denies any surgical history. She is prescribed levo-thyroxine 100 μg daily and albuterol as needed. She denies allergies to medications, tobacco or drug use, and drinks five glasses of wine per week. Her family history is notable for a mother who had menopause at age 51 and was diagnosed with ER/PR/HER2 negative breast cancer at age 80.

Evaluation of this patient includes:
- Complete patient history including:
- Menstrual cycle history.
- History and frequency of menopausal symptoms (hot flushes, vaginal dryness, sleep disturbances, mood changes).
- Family history.
- Complete physical exam including:

  – Vitals signs.
  – BMI calculation.
  – Skin and hair exam.
  – Thyroid exam.
  – Pelvic exam.

- Laboratory evaluation:

  – FSH—51 mIU/mL.
  – LH—38 mIU/mL.
  – Estradiol—20 pg/mL.
  – TSH—2.0 mIU/L.
  – Complete blood counts and metabolic panel—normal.

- Imaging evaluation:

  – Transvaginal ultrasound—small uterus with 2 mm endometrial stripe and bilateral small ovaries compatible with menopause.
  – Dual-energy X-ray Absorptiometry (DEXA)—no evidence of osteopenia or osteoporosis.

- Differential Diagnosis:

  – Menopause.
  – Anxiety.
  – Hypoglycemia.
  – Hyperthyroidism.
  – Flushing related to diet.
  – Carcinoid syndrome.
  – Malignancy (lymphoma, renal cell carcinoma, medullary thyroid carcinoma).
  – Infections (mycobacterial, bacterial, HIV, hepatitis C).
  – Substance withdrawal.
  – Systemic mast cell disease mastocytosis.
  – Pheochromocytoma.
  – Iatrogenic.

Diagnosis

Physical exam showed normal vital signs and a BMI of 27.5. There were no abnormalities in skin texture or hair thinning. Examination of the thyroid revealed no enlargement, nodularity, or tenderness. Pelvic exam revealed normal external female genitalia, mild vaginal mucosa atrophy, and an atrophic appearing cervix. There was no cervical motion tenderness, adnexal tenderness, or adnexal masses on bimanual exam. With laboratory results and imaging studies ruling out any other possible etiologies, a diagnosis of vasomotor symptoms associated with menopause was established.

Management

The treatment of vasomotor symptoms of menopause is recommended when symptoms are present and bothersome. For women with mild hot flushes that do not interfere with daily activity, treatment is not indicated. When symptoms become moderate (interfere somewhat with daily activity) or severe (daily activities cannot be performed), treatment is recommended. Associated symptoms such as sleep disturbances and mood changes should be assessed because the recommended therapy should target the symptoms which are most bothersome to the patient.

Behavioral changes should always be discussed with the patient first. This primarily includes dressing in layers which can be removed or added, using a fan or lowering the ambient temperature when possible, avoiding hot flush triggers (tobacco, spicy foods, and painful stimuli), and weight loss for overweight patients.

For patients with moderate or severe vasomotor symptoms, hormone therapy is recommended for women without contraindications [1]. Hormone therapy is generally safe for healthy women who are less than 10 years from the menopause or less than 60 years of age and do not have undiagnosed vaginal bleeding, a history of breast cancer, untreated endometrial cancer, cardiovascular disease, prior deep vein thrombosis, or active liver disease. Risk of developing cardiovascular disease and breast cancer should be assessed and hormone therapy should be avoided for patients that are considered high risk.

Once a patient decides to initiate hormone therapy, a strategy of using the lowest effective dose for the shortest time needed should be utilized. This can be done by starting at a low or moderate dose and titrating up or down as needed until symptom control is achieved. After a few years of treatment, attempts to wean the patient off of hormone therapy should be made every 1–2 years. This will help to minimize the risks associated with long-term hormone therapy use. Women without a uterus should be treated with estrogen therapy alone. Women with a uterus must be treated with estrogen and a progestin to oppose the effects of estrogen on the endometrium.

Common routes of estrogen therapy include oral, vaginal [2], and transdermal patch or gel. Progestin therapy is most often administered as oral micronized

progesterone. This formulation has a better side effect profile than medroxyprogesterone acetate. Progestins can be prescribed continuously or cyclically. Alternatively, progestins can be administered either trans-dermally or in the form of a progestin-containing intrauterine device (an off-label use for endometrial protection). Compounded bioidentical hormone therapy should never be used due a lack of regulation and concerns related to safety and efficacy with these products.

For women who have contraindications to hormone therapy or are not interested in taking hormone therapy, several alternative and effective medications can be used. Selective serotonin reuptake inhibitors (SSRI) and selective norepinephrine reuptake inhibitors (SNRI) are some of the best studied nonhormonal treatment options for vasomotor symptoms. Paroxetine [3], citalopram [4], escitalopram [5], venlafaxine, and desvenlafaxine are commonly used for this indication and have all been shown to effectively treat vasomotor symptoms. However, low-dose paroxetine 7.5 mg/day is the only FDA approved SSRI/SNRI for treatment of vasomotor symptoms. Compared to the dose used for treatment of depression and anxiety, a lower dose is usually able to achieve a response to vasomotor symptoms, which can be seen as early as 1 week after initiating treatment. SSRI/SNRIs are also a good treatment option for women who experience mood changes as a significant component of their menopausal symptoms.

Gabapentin is another nonhormonal medication which effectively treats vasomotor symptoms [6]. This medication has a sedating effect which can be an advantage when taken at night for women who experience nocturnal hot flashes and sleep disturbances as a significant component of their menopausal symptoms. High doses of gabapentin (900 mg 3×/day) have been shown to be as effective as hormonal therapy, however significant side effects limit the use of this dose. In practice, patients are often started on a low dose (100–400 mg nightly) with slow titration to achieve treatment effect while limiting side effects. Gabapentin can also be dosed up to 2–3 times/day if tolerated, however, nightly dosing is often sufficient to treat vasomotor symptoms.

Other options including clonidine, oxybutynin, and tibolone have been shown to improve vasomotor symptoms. These options are less commonly used because they are less effective than the options discussed above and they have a less favorable side effect profile. Importantly, placebo treatment has been shown to decrease vasomotor symptoms by 20–50%, which suggests there may be a mental health component to the frequency and intensity of symptoms experienced in some women. Other interventions which have been studied and shown to decrease vasomotor symptoms comparable to placebo include acupuncture, soy, and black cohosh.

Importantly, hormone therapy is associated with increased risks of thrombosis, stroke, and breast cancer. These risks are increased for women that use hormone therapy for a prolonged period of time or are older at the time of initiating therapy [7, 8]. While the absolute increase in the risks is small, it is important to appropriately counsel patients on the risks and benefits of these therapies. While young healthy women remain good candidates for hormone therapy, caution should be used when considering hormone therapy for women older than 60, women greater than 10 years from the menopause, and women with a moderate risk of breast cancer or cardiovascular disease.

A special population to consider is women with breast cancer. Hormone therapy is contraindicated for this population and therefore nonhormonal options should be used. For women currently taking Tamoxifen for endocrine therapy of disease, certain SSRIs should be avoided. Tamoxifen is converted to its active metabolite, endoxifen, by *CYP2D6*. Paroxetine and Fluoxetine are strong inhibitors of *CYP2D6* and theoretically could decrease the efficacy of tamoxifen therapy. While no evidence that taking these SSRIs concomitantly with tamoxifen increases breast cancer recurrence, it is generally best to avoid these two medications for patients taking tamoxifen. Other SSRI/SNRIs or gabapentin are acceptable alternatives.

This patient was deemed a good candidate for hormone therapy due to being both less than 60 years old and shorter than 10 years away from the menopause. She does not have any contraindications and her 5-year risk of developing breast cancer was calculated to be 2.4%. She was treated with initiation of transdermal estradiol 0.025 mg weekly and oral micronized progestin 200 mg/day for the first 12 days each month. After 1 month, she reported her daytime hot flushes had improved significantly, but that she continued to have night sweats and sleep interruptions. She was then initiated on gabapentin 100 mg nightly. After 4 weeks, she reported improvement in symptoms, now only waking from sleep with night sweats 2–3×/week. Her daytime drowsiness is now resolved and her focus at work is greatly improved. She also reported her motivation to exercise has increased and she is now back at the gym 3×/week, which she attributes to her improved sleep and decreased irritability throughout the day.

Discussion

The average age of menopause in the USA is 51. As the ovarian follicular pool decreases in the years leading up to menopause, there are less granulosa cells present in the ovary. These cells are responsible for secreting many of the regulators of the hypothalamic-pituitary axis including inhibin-B, inhibin-A, activin, anti-Mullerian hormone (AMH), and estradiol. AMH and inhibin-B regulate follicle stimulating hormone (FSH) secretion and the follicular response to this hormone. As AMH and Inhibin-B levels gradually decrease with the declining follicular pool, FSH levels prematurely rise to stimulate follicular growth and a resulting rise in estradiol levels. In the last 1–2 years before the menopause, a major decline in estradiol occurs until they plateau shortly after the menopause in a range between 10 and 20 pg/mL.

It is in response to the decreased circulating estrogen levels that menopausal symptoms arise. These symptoms are partially explained by a change in the thermoregulatory zone which can occur in response to decreased estrogen levels in women around the time of menopause. This change in estrogen levels can lead to altered regulation of central neurotransmitters resulting in a narrowed thermoregulatory zone in which sweating is triggered at a lower temperature. However, approximately 25% of women do not experience hot flushes after the menopause and therefore decreased estrogen levels alone do not fully account for vasomotor symptoms experienced by most women [9].

Sleep disturbances are also frequently reported after the menopause [10]. Nocturnal hot flushes are common and are often the primary cause of disordered sleep in these women. After initiation of treatment for vasomotor symptoms, sleep disturbances will usually improve or resolve. However, alternative explanations such as anxiety/depression and primary sleep disorders (obstructive sleep apnea, restless leg syndrome) are common in this population and should be explored.

Mood disorders are another common complaint of women presenting with menopausal symptoms. Evidence suggests that the menopausal transition itself is associated with an increased rate of depression even after adjusting for tobacco use, body mass index, hot flushes, and sleep disturbances [11]. Nevertheless, it is important to remember that many life changes may occur around the age of the menopause including changes in overall health, daily function, sexual dysfunction, family disruptions, and general changes associated with aging. Any of these changes alone can affect overall mood and could contribute to symptoms of anxiety or depression.

Finally, while this clinical scenario focused on vasomotor symptoms, another common presenting symptom of the menopausal transition is genitourinary syndrome of menopause [12]. Decreased estrogen levels lead to vulvovaginal atrophy which can lead to vaginal dryness, burning, irritation, urinary complaints and infections, and painful intercourse. When women present with both bothersome vasomotor symptoms and genitourinary syndrome of menopause, systemic hormone therapy is indicated and will often treat both complaints [13]. In patients who present with only genitourinary syndrome of menopause, local therapy is recommended. First line treatment options include vaginal moisturizers for daily use and vaginal lubricants for intercourse. If this treatment is inadequate, vaginal estrogen is an effective option which treats local symptoms without any significant change to the systemic estrogen levels and therefore can be an option for carefully selected patients who have contraindications to systemic hormone therapy [14]. Vaginal DHEA and ospemifene (selective vaginal estrogen receptor modulator) are alternative therapies which are FDA approved for the treatment of dyspareunia related to genitourinary syndrome of menopause.

References

1. Shifren JL, Crandall CJ, Manson JE. Menopausal hormone therapy. JAMA. 2019;321(24):2458–9.
2. Notelovitz M, Funk S, Nanavati N, Mazzeo M. Estradiol absorption from vaginal tablets in postmenopausal women. Obstet Gynecol. 2002;99(4):556–62.
3. Simon JA, Portman DJ, Kaunitz AM, Mekonnen H, Kazempour K, Bhaskar S, et al. Low-dose paroxetine 7.5 mg for menopausal vasomotor symptoms: two randomized controlled trials. Menopause. 2013;20(10):1027–35.
4. Barton DL, LaVasseur BI, Sloan JA, Stawis AN, Flynn KA, Dyar M, et al. Phase III, placebo-controlled trial of three doses of citalopram for the treatment of hot flashes: NCCTG trial N05C9. J Clin Oncol. 2010;28(20):3278–83.
5. Freeman EW, Guthrie KA, Caan B, Sternfeld B, Cohen LS, Joffe H, et al. Efficacy of Escitalopram for hot flashes in healthy menopausal women: a randomized controlled trial. JAMA. 2011;305(3):267–74.

6. Reddy SY, Warner H, Guttuso T, Messing S, DiGrazio W, Thornburg L, et al. Gabapentin, estrogen, and placebo for treating hot flushes: a randomized controlled trial. Obstet Gynecol. 2006;108(1):41–8.
7. Rossouw JE, Prentice RL, Manson JE, Wu L, Barad D, Barnabei VM, et al. Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. JAMA. 2007;297(13):1465–77.
8. Collaborative Group on Hormone Factors in Breast Cancer. Type and timing of menopausal hormone therapy and breast cancer risk: individual participant meta-analysis of the worldwide epidemiological evidence. Lancet. 2019;394(10204):1159–68.
9. Freedman RR. Menopausal hot flashes: mechanisms, endocrinology, treatment. J Steroid Biochem Mol Biol. 2014;142:115–20.
10. Erlik Y, Tataryn IV, Meldrum DR, Lomax P, Bajorek JG, Judd HL. Association of waking episodes with menopausal hot flushes. JAMA. 1981;245(17):1741–4.
11. Freeman EW, Sammel MD, Lin H, Nelson DB. Associations of hormones and menopausal status with depressed mood in women with no history of depression. Arch Gen Psychiatry. 2006;63(4):375–82.
12. Portman DJ, Gass MLS, Vulvovaginal Atrophy Terminology Consensus Conference Panel. Genitourinary syndrome of menopause: new terminology for vulvovaginal atrophy from the International Society for the Study of Women's Sexual Health and the North American Menopause Society. J Sex Med. 2014;11(12):2865–72.
13. Utian WH, Shoupe D, Bachmann G, Pinkerton JV, Pickar JH. Relief of vasomotor symptoms and vaginal atrophy with lower doses of conjugated equine estrogens and medroxyprogesterone acetate. Fertil Steril. 2001;75(6):1065–79.
14. Constantine GD, Simon JA, Pickar JH, Archer DF, Kushner H, Bernick B, et al. The REJOICE trial: a phase 3 randomized, controlled trial evaluating the safety and efficacy of a novel vaginal estradiol soft-gel capsule for symptomatic vulvar and vaginal atrophy. Menopause. 2017;24(4):409–16.

Index