Infertility is a common condition, affecting 10% to 15% of reproductive-aged couples. Defined as the inability to conceive after 1 year of intercourse, infertility can be subdivided into primary infertility (no prior pregnancies) and secondary infertility (infertility following at least 1 prior conception). The ability to conceive per month is defined as fecundability. Based on data from large population studies, the monthly probability of conceiving is 20% to 25% with approximately 50% of women pregnant at 3 months, 75% pregnant at 6 months, and 85% pregnant by 1 year. 

Although the prevalence of infertility is believed to have remained relatively stable during the past 40 years, the demand for infertility evaluation and treatment has increased considerably. Since the birth of the first in vitro fertilization (IVF) baby in July 1978, patients now have greater hope that medical intervention will help them achieve a successful pregnancy and are more likely to seek care. Furthermore, with their entrance into the workforce, women have intentionally delayed pregnancy and are now experiencing the decline in fertility rates associated with aging.

Achieving pregnancy is a complex process requiring multiple critical events. In the female, successful pregnancy requires ovulation, ovum pickup by a fallopian tube, fertilization, transport of the fertilized ovum through a patent fallopian tube into the uterus, and implantation into a receptive uterine cavity lined with healthy endometrium. From the male side, sperm of adequate number and quality must be deposited at the cervix near the time of ovulation. Problems may be encountered with each of these steps, resulting in infertility.

The frequency of each of the causes of infertility will vary depending on the patient population cared for by a specific clinical practice. In general, in cases where the cause of infertility can be traced, infertility is caused by female factors in one-third of cases, by male factors in one-third of cases, and by a combination of female and male factors in the remaining one-third of cases. Thus, it is imperative to evaluate both partners prior to instituting a treatment plan.

Keywords: fertility, andrology, semen analysis

After reading this article, readers should be able to discuss the general concepts of female and male reproductive physiology, list the 4 major areas examined during a fertility assessment, and describe diagnostic approaches, including major tests performed such as hormonal assays and semen analysis.

CE Update

Andrology and Fertility Assessment

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Abstract

Specialized clinical reproductive laboratories are an essential part of the infertility clinic, aiding in assessment and treatment of females and males. This update reviews female and male reproductive physiology, highlights important aspects of the fertility assessment, and details the diagnostic approach with an emphasis on testing performed in the clinical reproductive laboratory. Infertility is a common condition, affecting 10% to 15% of reproductive-aged couples. Although the prevalence of infertility is believed to have remained relatively stable during the past 40 years, the demand for infertility evaluation and treatment has increased considerably. In general, infertility is caused by female factors in one-third of cases, by male factors in one-third of cases, and by a combination of female and male factors in the remaining one-third of cases. Thus, it is imperative to evaluate both partners prior to instituting a treatment plan.

Keywords: fertility, andrology, semen analysis

Chemistry exam 21101 questions and corresponding answer form are located after this CE Update on page 53.

Abbreviations

IVF, in vitro fertilization; LH, luteinizing hormone; hCG, human chorionic gonadotropin; NSAIDs, nonsteroidal anti-inflammatory drugs; FAS, fetal alcohol syndrome; PID, pelvic inflammatory disease; PCOS, polycystic ovarian syndrome; POF, premature ovarian failure; TESA, testicular sperm aspiration; FSH, follicle-stimulating hormone; CBVAD, congenital bilateral absence of the vas deferens; HSG, hysterosalpingography; SIS, saline infusion sonogram; GnRH, gonadotropin releasing hormone; AMH, anti-Müllerian hormone; MIS, Müllerian inhibitory substance; ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay; P4, progesterone; DHEA-S, dehydroepiandrosterone; DHT, dihydrotestosterone; CASA, computer-assisted sperm analysis; WHO, World Health Organization; HOS, hypo-osmotic swelling; CCCT, Clomiphene Citrate Challenge Test; ICSI, intracytoplasmic sperm injection; HPG, hypothalamic-pituitary-gonadal

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In cases where the cause of infertility can be determined, one-third of the time it is due to male factor issues, one-third is due to tubal factors, and one-third is due to other issues such as ovulatory dysfunction, cervical factors, or a combination of factors. This highlights the importance of evaluating both partners prior to instituting a treatment plan. Figure 1 illustrates the incidences of various causes of infertility.

**Female Reproductive Physiology**

The classic menstrual cycle interval is 28 days with 4 to 5 days of bleeding and a blood loss of 40 mL to 100 mL. Considerable variability exists in these numbers with intervals ranging from 21 to 35 days and bleeding lasting from 2 to 7 days. The menstrual cycle is divided into the following 2 phases: the follicular phase and the luteal phase. The first day of bleeding is considered to be cycle day 1. Generation of a cyclic, controlled pattern of uterine bleeding requires precise temporal and quantitative regulation of a large number of reproductive hormones as illustrated in Figure 2.

The ovarian follicle consists of oocytes (eggs) with the following 2 types of surrounding cells: the adjacent granulosa cells and the more peripheral thecal cells. While a cohort of multiple follicles begins to develop, only 1 of the follicles, termed the dominant follicle, will proceed to ovulation. This growing follicle develops a large, fluid-filled cavity (the antrum) in which the oocyte is surrounded by specialized...
granulosa cells (the cumulus oophorus). In response to the luteinizing hormone (LH) surge, the follicle ruptures and the oocyte is extruded along with the attached granulosa cells. The remaining ovarian granulosa and thecal cells differentiate into the corpus luteum. Unless rescued by human chorionic gonadotropin (hCG) production (ie, pregnancy), the corpus luteum spontaneously regresses after 14 days. The endometrium cannot be maintained in the presence of sharply decreasing estrogen and progesterone (P4) secretion and is sloughed as menstrual bleeding.

Both the endocervical glands and the vaginal epithelium undergo cyclic changes as well. The endocervical glands secrete mucus serving multiple functions. Normally thick and impervious to sperm and ascending infection into the uterus, the mucus becomes thin and elastic (“spinnbarkeit,” literally stretchiness) due to the high estrogen levels at the time of ovulation. This midcycle mucus prevents entry of the nonsperm components of the semen into the uterus (ie, cellular debris, prostaglandins). In addition, the mucus forms channels that help direct sperm into the uterus and act as a reservoir for ongoing release during the next 24 to 72 hours, extending the fertile period. With the rise in P4 levels after ovulation, the quantity and elasticity of the mucus declines. These viscosity changes form the basis for simple, indirect tests of ovarian function that are useful both for natural family planning as well as timing intercourse for couples attempting conception.

**Male Reproductive Physiology**

Analogous to the ovary, the testes have 2 primary functions: the generation of mature germ cells (sperm) and the production of steroid hormones (primarily testosterone). Sperm production occurs in the seminiferous tubules with the aid of support cells known as Sertoli cells. Spermatic tubules occupy approximately 80% of testicular volume and also create a unique environment known as the blood-testes barrier. Differentiating germ cells may be recognized by the body as foreign and potentially antigenic, thus the body sequesters these cells from the blood system. The testes are unique in that they contain stem cells that are continuously producing mature germ cells throughout a man’s life. Through a continual process, the entire sequence of spermatogenesis from germ cell to mature spermatozoan requires approximately 90 days to complete.

Approximately 5% of total testicular volume is composed of Leydig cells, which are located in the interstitial tissue between the seminiferous tubules. Leydig cells are responsible for the synthesis of androgens from cholesterol. The process of testosterone synthesis in the testes is controlled by the hypothalamic-pituitary-testicular axis and is summarized in Figure 3. The proper intratesticular hormonal milieu is essential for normal spermatogenesis.

Human sperm are highly specialized cells with a single function: to deliver genetic material to the oocyte. Sperm lack many normal cellular organelles, including ribosomes, nucleoli, rough endoplasmic reticulum, and Golgi apparatus. Sperm consist of a head, midpiece, and flagellum or tail. The sperm head contains the densely packed genetic material (either an X or Y chromosome) and the acrosome. The acrosome is located at the top of the head and contains hydrolytic enzymes which, when released during the acrosome reaction, aid the sperm in penetrating the cumulus cells and zona pellucida surrounding the oocyte. The midpiece contains the sperm’s mitochondria supplying the energy necessary for motility. The sperm tail consists of microtubules allowing sperm to move. Fertility can be adversely affected if any of the sperm parts are damaged due to genetic mutations, hormonal imbalances, or environmental influences such as tobacco, sexual lubricants, saliva, alcohol, drugs, or excessive heat.

Spermatozoa are ejaculated through the urethra with secretions from accessory sex glands. The mixture of secretions and sperm is called semen. Laboratory measurement of certain seminal fluid components, such as fructose, are helpful in elucidating certain types of male infertility that may be due to blockages or other issues with the accessory sex glands (ie, seminal vesicles, prostate, bulbourethral glands, and epididymis).

**Fertility Evaluation**

The initial infertility evaluation consists of assessment of 1) history and physical exam of female and male partners; 2) reproductive tract anatomy; 3) ovulatory function; and 4) semen characteristics. The specifics regarding evaluation of each of these categories will be detailed in the following sections and are shown in Figure 4.

**History and Physical**

The infertile woman should be questioned regarding menstruation (frequency, duration, and recent changes in interval or duration), prior contraceptive use, coital frequency (it is important to ensure that lack of coitus is not the reason for infertility), and duration of infertility. Cyclic menstrual bleeding is strong evidence of ovulation. A prior pregnancy indicates the patient has ovulated and had patent fallopian tubes in the past. A history of abnormal Pap smears may be pertinent, particularly if the woman underwent cervical surgery that could impact cervical mucus quality.

A physical examination may also provide many clues to the cause of female infertility. The presence of significant acne or excess hair growth on the face or on the lower abdomen indicates the need to measure androgen levels. The pelvic exam may be particularly informative. Adequate estrogen production will be demonstrated by a moist, pink vagina and the presence of cervical mucus. Uterine fibroids will often be detected due to an enlarged or irregularly shaped uterus. An immobile or fixed uterus suggests the presence of pelvic scarring due to endometriosis or pelvic infection.

The male partner also must be questioned regarding his health and lifestyle. The testes are located out of the pelvis in order to optimize spermatogenesis, which requires temperatures slightly below body temperature. Illness with high fevers or chronic hot tub use can temporarily impair sperm quality. Erectile dysfunction or incomplete puberty may be associated with decreased testosterone levels.

On exam, typical male beard growth as well as the presence of axillary and pubic hair indicate the production of testosterone. The penile urethra should be at the tip of the glans to allow sperm to be deposited at the cervix. The testes should be at least 4 cm in length with a minimal volume of 20 mL. As the majority of testicular volume is provided by the
seminiferous tubules, decreased testicular volume is a strong predictor of abnormal spermatogenesis. Testicular cancer may present as infertility and be detected as a testicular mass. The epididymis and prostate glands should be soft and nontender to exclude chronic infection. Additionally, the pampiniform plexus of veins should be palpated for varicocele.5 Importantly, both vas deferens should be palpable as congenital bilateral absence of the vas deferens (CBAVD) is associated with mutation in the gene responsible for cystic fibrosis.6

The medical history for both partners should include questions about symptoms related to either hyperprolactinemia or thyroid disease, both of which can prevent normal ovulation and result in irregular or absent menstrual cycles. Thyroid disease may be manifested by changes in weight, energy levels, appetite, bowel habits, or sleep pattern. Elevated serum prolactin levels often lead to galactorrhea (milky breast discharge).

Medications should be noted, including over-the-counter medications, including nonsteroidal anti-inflammatory drugs (NSAIDs) that may adversely affect ovulation. Cigarette smoking negatively impacts fertility in women and men.7-11 Heavy alcohol intake decreases fertility in women and has been associated with a decrease in sperm counts and an increase in sexual dysfunction in men.12,13 Furthermore, excessive alcohol intake during pregnancy may result in fetal alcohol syndrome (FAS), which includes characteristic facial abnormalities, intellectual deficits, and behavioral abnormalities. Caffeine consumption has been linked to decreased fecundability and an increase in miscarriage rates.14-16 Illicit drugs also impact fertility rates. Marijuana suppresses the HPG axis in men and women, preventing egg and sperm production. Cocaine has been linked to impaired spermatogenesis.17,18

Sexually transmitted diseases or frequent infections of the epididymis or prostate gland may lead to obstruction of the vas deferens. A history of cryptorchidism, testicular trauma, or mumps as an adult can result in decreased spermatogenesis.19-23

Treatment with chemotherapy or local radiation therapy may have damaged the ovaries or spermatogonial stem cells. Hypertension, diabetes mellitus, and neurologic disorders may be associated with erectile dysfunction or retrograde ejaculation. The increasing use of anabolic steroids also decreases sperm production by suppressing the production of

Figure 3. The HPG axis plays a crucial role in female and male fertility. The hypothalamus produces Gonadotropin-releasing hormone (GnRH), which stimulates the pituitary to secrete the gonadotropins FSH and LH. In females, FSH stimulates the growth of ovarian follicles and the production of estrogen. Estrogen in turn helps prepare the uterus for a potential pregnancy. When estrogen levels peak, the “LH surge” causes ovulation and subsequent P4 secretion by the corpus luteum. If fertilization of the oocytes does not occur, P4 production ceases with regression of the corpus luteum and shedding of the uterine lining, and the cycle begins anew. In males, LH acts on the Leydig cells in the testes, allowing testosterone biosynthesis. Testosterone is necessary for normal sperm production.
intratesticular testosterone. Although the effects of many medications are reversible, anabolic steroid abuse may lead to long-lasting damage to testicular function.

Assessment of Reproductive Tract Anatomy

A wide variety of etiologies may result in obstruction of the fallopian tubes and/or pelvic adhesions (scarring), including pelvic infection, endometriosis, and prior pelvic surgery. A history of pelvic inflammatory disease (PID) is highly suspicious for damage to the fallopian tubes or the presence of pelvic adhesions or both. In the United States, the most common causes of tubal disease are infection with *Chlamydia trachomatis* or *Neisseria gonorrhoeae*, whereas tuberculosis is a common cause of tubal and intrauterine disease in countries with endemic infection. Severe pelvic adhesions can also develop due to endometriosis (the presence of endometrial tissue outside of the uterine cavity), certain surgical procedures such as appendectomy, and a history of ectopic pregnancy.

Uterine anomalies, whether inherited or acquired, can also be a significant cause of female infertility; however, no uterine abnormalities currently require laboratory evaluation. Abnormalities in cervical mucus production are most frequently observed in women who have been surgically treated for an abnormal Pap smear by removal of the superficial cells of the cervix. It has also been proposed that cervical infection can negatively impact mucus quality; however, these data remain controversial.

Assessment of Ovulatory Function

Oocyte development and ovulation require hormonal communication between the hypothalamus, anterior pituitary gland, and ovaries. Lifestyle issues, such as excessive exercise, eating disorders, or stress, may perturb normal hypothalamic function resulting in anovulation. Polycystic ovarian syndrome (PCOS) is the most common cause of anovulation, occurring in 5% to 10% of reproductive-aged women. This disorder is characterized by the presence of irregular menses since puberty, excess androgen production by the ovaries, and multiple small cysts within the ovaries, which have arrested...
in development. Although variable, these patients tend to be overweight with glucose intolerance (pre-diabetes mellitus) and elevated serum lipid levels. Thyroid disease and hyperprolactinemia may also contribute to menstrual disturbances. Suspicion of any of these clinical disorders will result in requests for hormonal measurements from the clinical laboratory.

Premature ovarian failure (POF) is a condition found in approximately 1% of the female population and is defined as women less than 40 years old with persistent amenorrhea and elevated gonadotropin levels. Premature ovarian failure is often idiopathic but may be due to autoimmune causes in up to 20% of patients. If POF is diagnosed prior to age 30, a karyotype is warranted to rule out the presence of a Y-chromosome mosaicism, in which case the ovary should be removed to prevent development of a tumor. Chemotherapy and radiation therapy for cancer as well as surgical removal of the ovaries can also cause POF. Women with POF who wish to become pregnant will likely need to employ a donor oocyte.

There is a clear inverse relationship between female age and fertility. The rate of follicular loss and age at menopause varies between women and is likely determined by genetic and environmental factors. It is generally impossible to predict the onset of menopause; therefore, testing is indicated to determine an individual woman’s fertility status.

**Assessment of Semen Characteristics**

The causes of male infertility can be categorized as abnormalities of sperm production (number, motility, and morphology), obstructions or functional abnormalities, endocrine disturbances, or anatomic or genetic defects.

Deficiencies in sperm production are a major cause of male fertility issues. The most severe cases of male infertility involve men who have no sperm present in the ejaculate, termed azoospermia. A diagnosis of azoospermia occurs only if no sperm is found in a minimum of 2 samples collected at least 1 month apart that have been concentrated by centrifugation. Azoospermia may be obstructive or non-obstructive. Obstructions may occur anywhere along the genital tract but are most common in the epididymis. Laboratory testing for chemical biomarkers such as fructose is recommended for patients who present with <1 million sperm/mL to determine if there is an obstruction in the seminal vesicles. Surgical procedures such as testicular sperm aspiration (TESA) may be performed to retrieve sperm blocked by an obstruction.

Other deficiencies in sperm production such as low sperm count (oligozoospermia), low motility (asthenozoospermia), and/or morphological defects (teratozoospermia) can also be discovered during a semen analysis. Lifestyle changes such as quitting smoking, drinking, and/or drug use and keeping the testes cool by refraining from activities such as hot tub use or excessive bicycling or running may help. However, as long as some living sperm are present, the sample may be used in conjunction with IVF treatment.

The male ejaculatory system may be functionally or anatomically abnormal. Developmental anomalies of the penis, such as hypospadias, may prevent normal deposition of sperm in the vaginal vault. Retrograde ejaculation can be considered a functional cause of obstruction of the vas deferens. In this disorder, the sphincter between the bladder and the vas deferens does not close properly at the time of ejaculation, allowing semen to be propelled into the bladder rather than down the urethra. Prostate surgery or diabetes mellitus with its associated neuropathy are the 2 most common etiologies for retrograde ejaculation. Patients with spinal cord injury may not be able to ejaculate spontaneously, but ejaculation can often be achieved with electroejaculation.

The presence of a varicocele is associated with decreased fertility. Varicoceles consist of dilation of the veins draining the testes, also called the pampiniform plexus, and raising scrotal temperatures. The majority of varicoceles are on the left side, due to differences in drainage pattern on this side of the body; however, they may be bilateral. In general, larger varicoceles are associated with poorer semen characteristics. Data have also suggested that varicoceles may also cause progressive declines in semen parameters, implying the need for early surgical correction. It must be noted that substantial controversy exists regarding the impact of varicoceles on pregnancy rates or the benefits of varicocelectomy.5,26-30

A history of cryptorchidism (failure of proper descent of the testicles from the pelvis into the scrotum) may result in male infertility. The effects on spermatogenesis are believed to be due to increased heat exposure during testicular development. It is also possible that the genes directing testicular descent are also important for sperm production, and therefore mutations in these genes may have a negative impact through multiple mechanisms. Testicular torsion or trauma also may result in infertility.

Hormonal disorders can also affect male fertility. In cases of azoosperma and/or small testicular size, assays for the gonadotropins follicle-stimulating hormone (FSH) and LH as well as testosterone should be performed. Hypogonadotropic hypogonadism is characterized by low gonadotropin and low testosterone levels. This condition is treatable, but the process is often a long and difficult regime of gonadotropin replacement therapy. Hypogonadotropic hypogonadism, in which gonadotropin levels are elevated but testosterone remains low, has a poorer prognosis in that it usually suggests low- or non-functioning gonads. This is parallel to POF in women.

Several genetic disorders have been linked to reduced male fertility. One of the most commonly observed is CBAVD. Congenital bilateral absence of the vas deferens occurs due to a mutation in the CFTR gene (the gene responsible for cystic fibrosis) in which the vas deferens, tubes connecting the testicles to the penis, do not form during gestation. Therefore, affected patients present with azoospermia although their testicles usually produce sperm. These patients may have mild or no other sequelae. Klinefelter’s syndrome is another relatively common genetic defect resulting in infertility. Klinefelter’s males carry an extra chromosome (47,XXX or 47,XY) making it difficult for their germ cells to undergo meiosis, resulting in absent or severely defective sperm. Less common genetic defects include Kartagener’s syndrome, or immotile cilia syndrome, where affected men produce immotile sperm due to lack of functional cilia, and Young’s syndrome, which causes azoospermia due to functional obstructions by extremely viscous seminal fluid components.

**Diagnostic Approach**

The initial infertility evaluation involves a number of laboratory tests to assist in the assessment of reproductive tract anatomy, ovulatory function, and semen characteristics. The
specifics regarding evaluation of each of these categories will be detailed in the following section and are summarized in Figure 4.

1. Rule out pregnancy. Prior to any assessment of the reproductive tract anatomy, it is prudent to rule out a pre-existing early pregnancy. Human chorionic gonadotropin is a glycoprotein hormone secreted by placental tissue and found in blood and urine almost exclusively in pregnancy. Home pregnancy tests qualitatively determine pregnancy by measuring hCG in urine. Quantitative hCG values from blood serum can be performed by a number of commercial immunoassays. Serum or urine specimens are analyzed by a number of FDA-approved commercial analyzers with proper high and low controls. Males and non-pregnant females should have hCG levels lower than 5 mIU/mL. Pregnant women should have detectable circulating hCG levels by 8-10 days post-conception. These levels should roughly double every 2 days until peak levels are attained around 12 weeks gestation, at which time hCG levels will drop for the remainder of pregnancy. Multiple gestations will often have higher hCG levels, while ectopic pregnancies (pregnancies occurring outside of the uterus) and pregnancies ending in spontaneous abortion will often demonstrate lower hCG levels. It should be noted that there may be some disparity between commercial analyzers depending on which part of the hCG molecule the assay targets. If monitoring a pregnancy over time, it is best to have samples run on the same type of analyzer for optimal interpretation.

2. Visualize reproductive tract anatomy. Hysterosalpingography (HSG), radiographic evaluation using contrast media, may be used to evaluate the size and shape of the uterine cavity in addition to determining whether the fallopian tubes are patent. Alternatively, saline may be infused into the uterine cavity during transvaginal ultrasound (saline infusion sonogram, SIS).

3. Assess ovarian reserve.
   (a) Gonadotropins. Both FSH and LH are glycoprotein hormones secreted by the gonadotrope cells of the anterior pituitary under the control of the hypothalamic gonadotropin releasing hormone (GnRH). Follicle-stimulating hormone and LH are required for normal sexual function in females and males. Gonadotropins are given exogenously to women in assisted reproduction cycles as it initiates the response to stimulation. Inhibin A or inhibin B by the ovarian luteal cells and granulosa cells. Anti-Müllerian hormone levels tend to decrease with age and have a positive correlation to ovarian antral follicle count at ultrasound. Low AMH levels indicate a diminished ovarian reserve and generally forecast poor stimulation during IVF cycles. Anti-Müllerian hormone levels do not vary significantly throughout the menstrual cycle. Serum specimens are used to measure AMH levels in an enzyme-linked immunosorbent assay (ELISA) based system. Anti-Müllerian hormone is a relatively new marker for ovarian reserve, and therefore, the reference ranges should be viewed as a continuum rather than as absolute cut-offs. Serum AMH levels >1.5 ng/mL are considered normal while levels below 0.8 ng/mL are associated with increased IVF cancellation rates due to poor ovarian response to stimulation.

   (b) Estradiol. Estradiol is a steroid hormone circulating in free and bound forms, with free estradiol being more biologically active. Estradiol and other estrogen derivatives, such as estrone and estriol, are secreted at varying rates throughout the menstrual cycle. Estradiol plays a crucial role in cervical mucus production, endometrial proliferation to aid in embryo implantation, and induction of the LH surge. Thus, measurement of estradiol yields useful information for the infertility workup and for monitoring of assisted reproduction cycles. Serum estradiol levels can be measured by a number of commercial immunoassay methods. Estradiol levels may be obtained at the time of FSH measurement in order to decrease the incidence of false-negative results with FSH measurement alone. Somewhat paradoxically, estrogen levels in older women are elevated early in the cycle due to increased stimulation of ovarian steroidogenesis by elevated FSH levels. A cycle day 3 estradiol level of >80 pg/mL is considered abnormal and is consistent with declining ovarian function. For monitoring of assisted reproduction cycles, estradiol levels are frequently measured because circulating estradiol levels correlate with follicular size and number, and estradiol levels help to calibrate the dose of exogenous gonadotropins given to each patient through her cycle.

   (c) Anti-Müllerian hormone (AMH), which is also sometimes called Müllerian inhibitory substance (MIS), is a dimeric glycoprotein hormone secreted by ovarian granulosa cells. Anti-Müllerian hormone levels tend to decrease with age and have a positive correlation to ovarian antral follicle count at ultrasound. Low AMH levels indicate a diminished ovarian reserve and generally forecast poor stimulation during IVF cycles. Anti-Müllerian hormone levels do not vary significantly throughout the menstrual cycle. Serum specimens are used to measure AMH levels in an enzyme-linked immunosorbent assay (ELISA) based system. Anti-Müllerian hormone is a relatively new marker for ovarian reserve, and therefore, the reference ranges should be viewed as a continuum rather than as absolute cut-offs. Serum AMH levels >1.5 ng/mL are considered normal while levels below 0.8 ng/mL are associated with increased IVF cancellation rates due to poor ovarian response to stimulation.

   (d) Inhibin. The peptide inhibin is secreted as either inhibin A or inhibin B by the ovarian luteal cells and granulosa cells, respectively. Inhibin blunts FSH production by the anterior pituitary gland. Although not routinely performed, inhibin B may be used, in conjunction with FSH and estradiol measurements, to predict follicular health. Inhibin is measured in serum using radioimmunoassay (RIA) or ELISA.

   For women, the normal range for a cycle day 3 sample is 45-200 pg/mL. The Inhibin B test is not widely performed in fertility clinics as current published data does not overwhelmingly support this test over measurement of a cycle day 3 serum FSH.

4. Check for ovulation.
   (a) Mid-luteal P4. Progesterone is a steroid hormone crucial for preparation for and maintenance of pregnancy. Circulating P4 levels are low during the follicular phase of the menstrual cycle and sharply increase during the luteal phase, with peak levels occurring 5-10 days after the LH surge. A cycle day 21 P4 measurement is usually a good indicator of whether ovulation has occurred. Progesterone levels during the follicular phase are typically <2 ng/mL. Midluteal P4
concentrations of at least 3 ng/mL are considered ovulatory, and levels of >10 ng/mL are predictive of higher pregnancy rates than values falling under this threshold.

(b) Androgens. Androgens including total testosterone, dehydroepiandrosterone (DHEA-S), and 17-hydroxyprogesterone may be included in a fertility workup if the woman presents with hirsutism (excess hair growth) or extensive acne. Dihydrotestosterone (DHT) may be measured to evaluate more severe signs of androgen excess (true virilization) including balding and deepening of the voice. Excess androgen production in women is most commonly due to the presence of polycystic ovarian syndrome (PCOS). Polycystic ovarian syndrome is associated with absent or irregular ovulation and, as a result, infertility. Moderately elevated total testosterone levels (50-60 ng/dL) are consistent with the diagnosis of PCOS, but highly elevated levels (>200 ng/dL) may indicate more serious issues, such as an ovarian tumor requiring imaging studies. Elevated serum androgen levels may also be caused by an adrenal tumor or by mutation of 1 of the steroidogenic enzymes, similar to what occurs with congenital adrenal hyperplasia. Controversy exists regarding the use of commercial automated chemiluminescent methods to analyze testosterone levels in human serum as each method contains inherent errors. This arises, in part, due to the fact that the normal physiological range for testosterone in men and women differs sharply and some analyzers may not reflect this in their reference limits. Therefore, it is strongly advised that laboratories develop their own reference intervals for these tests.

5. Complete Semen Analysis. A complete semen analysis including measurement of semen volume, sperm motility, sperm count, and sperm morphology is the core test in the evaluation of male fertility status. Semen analyses can be performed manually or with the aid of a computer-assisted sperm analysis (CASA) program. Human semen is analyzed, preferably collected by masturbation without the aid of lubricants or saliva, from an individual who has abstained from ejaculation for 2-7 days prior to the analysis. The specimen should be analyzed within 1 hour of collection.

An initial macroscopic examination of the specimen checks the general appearance of the specimen (homogenous, grey-opalescent), whether the specimen has liquefied (which normally will occur within 15 minutes of ejaculation but may take up to 60 minutes), volume (in mL), and viscosity (how easily the specimen is to pipet). The specimen is then observed under phase contrast microscopy to estimate sperm count (in millions/mL) and percent motility. Sperm motility is measured on a simple grading system (a-d) where “a” is rapid progressive motility, “b” is slow or sluggish motility, “c” is non-progressive motility or ‘twitching,’ and “d” is immotility. Also, morphology assessment is often performed by making a smear of the semen on a glass slide and staining the slide to assess the sperm’s morphological features. The Papanicolaou stain is the gold standard for morphology assessment, but many laboratories will use a rapid staining method such as Diff-Quik. There are 2 accepted grading systems for sperm morphology, World Health Organization (WHO) and Kruger strict criteria, both of which involve examination of individual sperm for head, midpiece, and/or tail defects. See Figure 5 for WHO guidelines for normal sperm parameters. Proper quality control is essential for performance of a semen analysis as intra-technician and intra-laboratory variabilities are common.

Additionally, the following tests may be performed if indicated.

(a) Sperm Viability Test. If the percentage of immotile spermatozoa exceeds 50%, the WHO recommends a sperm viability test be performed to determine if the sperm are alive but immotile or dead.

Viability is determined in a semen sample using the dye exclusion or hypo-osmotic swelling (HOS) test. For the dye exclusion test, eosin-nigrosin dye is commonly used. A drop of liquefied semen is first mixed with 2 drops of 1% (weight/volume) eosin Y in distilled water (C.I. 45380), followed by 3 drops of 10% (weight/volume) nigrosin solution in distilled water. Since this dye is a variable mixture of complex compounds of unknown constitution, quantitative determination of the dye content is impossible (C.I. 50420). The mixture is then smeared on a slide, which is allowed to air dry prior to being examined under oil immersion with a light microscope.

The HOS test: A solution of sodium citrate dehydrate and fructose is mixed with liquefied semen and incubated at 37°C for 30-120 minutes. Spermatozoa are then examined under a phase-contrast microscope for changes in tail shape indicating swelling. A subset of sperm will have curled tails prior to HOS treatment; therefore, it is critical to determine the baseline frequency of sperm with this characteristic. Final results should be calculated as the percentage of untreated sperm with curled tails after sodium citrate treatment minus the percentage curled prior to treatment.

For both procedures, the sum of dead and motile spermatozoa should not exceed 100%. In the dye exclusion test, live spermatozoa will be white as they can exclude the eosin dye, and dead spermatozoa will stain pinkish red as they are unable to exclude the dye. The nigrosin stain provides a dark background, making it easier to read the slide. For the HOS test, greater than 60% of spermatozoa should undergo tail swelling in normal specimens. A specimen is considered abnormal if <50% of the specimen exhibit tail swelling.

(b) Fructose evaluation in seminal plasma. The presence of fructose in seminal plasma indicates intact secretory function of the seminal vesicles. The fructose assay is colorimetric and can be quantitative or qualitative. For a qualitative fructose test, a positive control (fructose solution), a negative control (water), and a portion of the semen sample to be tested are each mixed with resorcinol, vortexed, and heated for 10-15 minutes in a boiling water bath, until the positive control changes from clear to bright orange in color. An orange-colored sample is positive, indicating the presence of fructose provided by the seminal vesicle. A colorless-clear sample is negative, indicating a lack of seminal vesicle secretions due to a block or lack of formation of either the seminal vesicle ducts or the vas deferens. Mutations in the cystic fibrosis gene frequently result in CBAVD and, thereby, a negative fructose test. Genetic testing can be performed to evaluate for this possibility. As this is a qualitative test, it is important to always run positive and negative controls with each specimen and ensure they perform as expected prior to reporting results. Typically, a fructose test is only performed once on a patient if positive, even if the patient returns for a follow-up semen analysis.

(c) White cell detection in semen. When a semen sample is observed to contain greater than 5 million “round cells” per mL of sample, differentiation of round cells from immature sperm cells as well as differentiation among various types of round cells may be desired. Presence of excessive leukocytes in semen (leukocytospermia) is associated with possible infection and poor sperm quality. Histochemistry is performed to identify peroxidase in cells (a marker for leukocytes). In
general, a peroxidase stain solution is mixed with a liquefied semen sample, and the number of peroxidase-positive (brown-stained) and peroxidase-negative (unstained) cells are counted. Alternatively, immunocytochemistry with specific monoclonal antibodies can be performed to differentiate between various types of leukocytes. If high numbers of leukocytes are found (>1 million/mL), further microbiological testing should be performed to determine if there is an accessory gland infection (eg, infection of the epididymis).

Ancillary Tests

Clomiphene Citrate Challenge Test (CCCT). Clomiphene citrate is a non-steroidal estrogen receptor modulator thought to block the negative feedback inhibition of estrogen on secreted FSH. The CCCT is believed to be a more sensitive indicator of diminished ovarian reserve than measurement of “unstimulated” hormone levels. For the CCCT, estradiol and FSH levels are measured on cycle day 3, 100 mg of clomiphene citrate is taken orally on days 5-9, and FSH is re-measured on cycle day 10. Elevated FSH levels at either time point is indicative of diminished ovarian reserve. This test is normally considered for women >35 years old or who have a history of chemotherapy or radiation or a family history of early menopause.

Post-coital test. This test is performed to evaluate cervical mucus during ovulation (35, 36). A couple is instructed to have intercourse on the day of ovulation, the female partner is seen in the clinic a few hours later, and a sample of cervical mucus is obtained. Ideally the mucus should be copious, stretchy, and clear with minimal other cell types. At least 5 motile sperm per high-powered microscope field should be present. This test is now rarely performed due to lack of standardization and poor predictive value for pregnancy, but it should be considered for couples who would not consider intrauterine insemination or do not have intrauterine insemination readily available.

Antisperm antibodies. Antisperm antibodies may be detected in up to 10% of the male population, but they are usually more prevalent following a situation where the blood-testes barrier has been breached such as vasectomy, testicular torsion, or testicular biopsy. Antisperm antibodies are not normally tested routinely as part of an infertility evaluation unless the male partner has a clear risk factor for the presence of these antibodies. The most commonly used assay contains immunobeads specific for IgG or IgA that are mixed with a sperm sample. The beads will bind to sperm that have antibodies present on their surface. Antisperm antibody testing is principally performed in men who will be undergoing IVF as fertilization rates may be improved by using intracytoplasmic sperm injection (ICSI) in an antibody-positive patient.

Genetic testing. Karyotype testing should be obtained on all azoospermic and severely oligospermic men. Although genetic abnormalities cannot be corrected, they may have implications for the health of the patient or their offspring and therefore, should be pursued when indicated by poor semen analysis results.

Course and Treatment

Evaluation for the causes of infertility will almost always include testing provided by clinical laboratories in the areas of hormone level assessment and semen analysis. Treatment options can be simplified as “get more eggs, get more sperm, or get the sperm closer to the eggs.” An increased number of mature oocytes (eggs) can be obtained by ovulation induction medications such as oral clomiphene citrate or gonadotropin injections. Higher number and quality sperm may be obtained, as examples, by treating genitourinary infections or by varicocele repair. Fertilization rates may be improved by intrauterine insemination or by IVF, including ICSI. With careful testing and focused intervention, the majority of infertile couples will be able to achieve their goal of having a child.


