Diagnostic evaluation of the infertile male: a committee opinion

Practice Committee of the American Society for Reproductive Medicine
American Society for Reproductive Medicine, Birmingham, Alabama

The purpose of this ASRM Practice Committee report is to provide clinicians with principles and strategies for the evaluation of couples with male infertility problems. This revised document replaces the document of the same name, last published in 2012 (Fertil Steril 2012;98:294–301). (Fertil Steril® 2015;100:1–41. ©2015 by American Society for Reproductive Medicine.)

INDICATIONS FOR EVALUATION
Evaluation for infertility is indicated for couples who fail to achieve a successful pregnancy after ≥12 months of regular unprotected intercourse. Earlier evaluation and treatment may be justified, based on medical history and physical findings and is warranted after 6 months for couples in which the female partner is >35 years old. Men having concerns about their future fertility also merit evaluation.

At a minimum, the initial screening evaluation of the male partner of an infertile couple should include a reproductive history and analysis of at least one semen sample. If the initial evaluation is abnormal, then referral to someone experienced in male reproduction is recommended.

Reproductive History
The reproductive history should include: 1) coital frequency and timing; 2) duration of infertility and previous fertility; 3) childhood illnesses and developmental history; 4) systemic medical illnesses (such as diabetes mellitus and upper respiratory diseases); 5) previous surgery; 6) medications and allergies; 7) sexual history (including sexually transmitted infections); and 8) exposures to gonadotoxins (including environmental and chemical toxins and heat). Previous fertility does not exclude the possibility of a newly acquired, secondary, male infertility factor. Evaluation is the same for men with primary infertility (never having fathered a pregnancy) and secondary infertility (having previously fathered a pregnancy).

Semen Analysis
Semen analysis is the cornerstone of the laboratory evaluation of the
infertile man and helps to define the severity of the male factor. Physicians should provide patients with standardized instructions for semen collection, including a defined pre-test abstinence interval of 2–5 days. Although a standard duration of abstinence is important for evaluation of semen parameters, some men with severe oligozoospermia can have equal or better sperm concentration with a short (hours) period of abstinence, supporting the potential use of multiple semen analyses during assisted reproductive technology treatment cycles (5–7). Semen can be collected by means of masturbation into a specimen cup or by intercourse with the use of special semen collection condoms that do not contain substances toxic to sperm. Ideally, the specimen should be collected at the laboratory. If collected at home, the specimen should be kept at room or body temperature during transport and examined in the laboratory within 1 hour of collection. To ensure accurate results, the laboratory should have a quality control program for semen analysis that conforms to the standards outlined in the Clinical Laboratory Improvement Amendments (CLIA); additional information including proficiency testing can be found on the CLIA website (8).

The semen analysis provides information on semen volume as well as sperm concentration, motility, and morphology (Table 1) (9). Methods for semen analysis are discussed in many textbooks, and detailed laboratory protocols have been published by the World Health Organization (WHO) (10). The diagnosis of azoospermia can be established only after the specimen is centrifuged (preferably at 3,000g) for 15 minutes and the pellet is examined. The current WHO criteria for evaluating sperm morphology (10) are similar to the “strict criteria” described by Kruger (Tygerberg) (11, 12), in that relatively few sperm are classified as having normal morphology, even in semen obtained from fertile men. Strict sperm morphology has been used to identify couples at risk for poor or failed fertilization with the use of standard in vitro fertilization (IVF) techniques (11) and thus to identify those who may be candidates for intracytoplasmic sperm injection (ICSI) (13). However, the value and necessity for ICSI in those having isolated abnormalities in strict morphology has been questioned (14).

### TABLE 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference value</th>
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<tbody>
<tr>
<td>On at least two occasions</td>
<td></td>
</tr>
<tr>
<td>Ejaculate volume</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>pH</td>
<td>7.2</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>$15 \times 10^6$ spermatozoal/mL</td>
</tr>
<tr>
<td>Total sperm number</td>
<td>$39 \times 10^6$ spermatozoal/ejaculate</td>
</tr>
<tr>
<td>Percentage motility</td>
<td>40%</td>
</tr>
<tr>
<td>Forward progression</td>
<td>32%</td>
</tr>
<tr>
<td>Normal morphology</td>
<td>4% normal</td>
</tr>
<tr>
<td>And</td>
<td></td>
</tr>
<tr>
<td>Sperm agglutination</td>
<td>Absent</td>
</tr>
<tr>
<td>Viscosity</td>
<td>$\leq 2$ cm thread after liquefaction</td>
</tr>
</tbody>
</table>

Note: Data from World Health Organization, 2010 (10).

Clinical reference ranges have been established for sperm concentration, motility, and morphology to help classify men as fertile or subfertile (15). The semen parameters of men with documented fertility have been compared with those of infertile men among couples participating in a clinical trial of superovulation and intrauterine insemination (IUI). Sperm parameters that predicted male fertility were sperm concentration $>48$ million/mL, sperm motility $>63\%$, and sperm morphology $>12\%$ normal (strict criteria). Parameters that predicted male subfertility were sperm concentration $<13.5$ million sperm/mL, sperm motility $<32\%$, and sperm morphology $<9\%$ normal. Values between the fertile and subfertile thresholds were considered to be “indeterminate” (16). Although each sperm parameter could predict fertility and subfertility, none was a powerful discriminator. It is important to emphasize that normal reference values for semen parameters do not reflect normal sperm concentration in the general population, nor do they equate with the minimum values required for conception; men with semen variables outside the reference ranges may be fertile, and conversely, men having values within the reference range may still be infertile.

**COMPONENTS OF A COMPLETE EVALUATION FOR MALE INFERTILITY**

When the initial screening evaluation reveals an abnormal male reproductive history or demonstrates abnormal semen parameters, a thorough evaluation by a urologist or other specialist in male reproduction is indicated. More detailed evaluation of the male partner should be considered also in couples with unexplained infertility and those who remain infertile after successful treatment of identified female infertility factors.

The more thorough evaluation for male infertility should expand on the screening evaluation by including a complete medical history and physical examination performed by a urologist or other specialist in male reproduction. Based on the results obtained, additional tests and procedures may be recommended, including serial semen analyses, endocrine evaluation, post-ejaculatory urinalysis, ultrasonography, specialized tests on semen and sperm, and genetic screening.

**Medical History**

The patient’s medical history can identify risk factors and behaviors or lifestyles that could have significant impact on male infertility. In addition to all of the elements of the reproductive history described above, the medical history should be expanded to include: 1) a complete review of systems; 2) family reproductive history; and 3) a detailed social history, including any past or current use of anabolic steroids, recreational drugs, tobacco, and alcohol.

**Physical Examination**

A general physical examination is an integral part of the evaluation of infertile men. Particular attention should be directed to the genitalia, including: 1) examination of the penis, noting the location of the urethral meatus; 2) palpation and
measurement of the testes; 3) the presence and consistency of both vasa and epididymides; 4) the presence or absence of a varicocele; 5) secondary sex characteristics, including body habitus, hair distribution, and breast development; and 6) digital rectal examination where indicated. The diagnosis of congenital bilateral absence of the vasa deferentia (CBAVD) is established by physical examination; scrotal exploration is unnecessary.

OTHER PROCEDURES AND TESTS FOR ASSESSING MALE INFERTILITY

Endocrine Evaluation

Hormonal abnormalities of the hypothalamic-pituitary-testicular axis are well recognized, but uncommon, causes of male infertility. Endocrine disorders are extremely uncommon in men with normal semen parameters.

An endocrine evaluation is indicated for men having: 1) abnormal semen parameters, particularly when the sperm concentration is < 10 million/mL; 2) impaired sexual function; or 3) other clinical findings that suggest a specific endocrinopathy. Some experts think that all infertile men merit an endocrine evaluation, but there is no established consensus of opinion. The minimum initial hormonal evaluation should include measurement of serum FSH and total testosterone (T) concentrations. When the total T level is low (< 300 ng/mL), more extensive evaluation is indicated and should include a second early morning measurement of total T and measurements of serum free testosterone (Tf), LH, and prolactin (PRL). Although serum gonadotropin concentrations vary because they are secreted in a pulsatile manner, a single measurement of serum free testosterone (Tf), LH, and prolactin (PRL) concentrations helps to provide an understanding of the source of abnormal total T levels (Table 2).

Whereas many men with abnormal spermatogenesis have a normal serum FSH, but a marked elevation of serum FSH is clearly indicative of an abnormality in spermatogenesis. In men with low ejaculate volume and oligozoospermia, “significant numbers” of sperm must be observed to support the diagnosis of retrograde ejaculation; there is no consensus of expert opinion on the minimum number required (18).

Ultrasonography

Because nearly the entire male genital tract can be imaged easily and accurately, ultrasonography is a useful tool for detecting abnormalities of the male genital tract that may adversely affect fertility. However, ultrasonography is indicated for only a minority of infertile male patients.

Transrectal ultrasonography. Normal seminal vesicles are usually < 1.5 cm in anteroposterior diameter (19). Transrectal ultrasonography (TRUS) revealing dilated seminal vesicles or ejaculatory ducts and/or midline cystic prostatic structures suggests, but does not by itself establish, the diagnosis of complete or partial ejaculatory duct obstruction (20). Affected men typically produce a low-volume acidic ejaculate containing no sperm or fructose. Men with CBAVD may exhibit similar findings because they often have absent or atrophic seminal vesicles. Men with partial ejaculatory duct obstruction often, but not always, exhibit low semen volume, oligoasthenospermia, and poor progressive motility. Some experts recommend routine TRUS for oligospermic men having low-volume ejaculates, palpable vasa, and normal

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>FSH</th>
<th>LH</th>
<th>T</th>
<th>PRL</th>
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<tbody>
<tr>
<td>Normal spermatogenesis</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Hypogonadotropic hypogonadism</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Normal</td>
</tr>
<tr>
<td>Abnormal spermatogenesisa</td>
<td>High/Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Complete testicular failure/hypogonadotropic hypogonadism</td>
<td>High/Normal</td>
<td>High</td>
<td>Normal/low</td>
<td>Normal</td>
</tr>
<tr>
<td>PRL-secreting pituitary tumor</td>
<td>Normal/low</td>
<td>Normal/low</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

*Many men with abnormal spermatogenesis have a normal serum FSH, but a marked elevation of serum FSH is clearly indicative of an abnormality in spermatogenesis.

testicular size with normal serum T, although there is no consensus on this.

**Scrotal ultrasonography.** Careful physical examination can identify most scrotal pathology, including varicoceles, spermatoceles, absent vasa, epididymal induration, and testicular masses. Scrotal ultrasonography can identify occult varicoceles that are not palpable, but such lesions have no demonstrated clinical significance [21]. Scrotal ultrasonography can be helpful for better defining vague or ambiguous physical examination findings or abnormalities (including apparent masses) and can be performed in men having tests located in the upper scrotum, a small scrotal sac, or other anatomy that hinders physical examination. Scrotal ultrasonography should also be considered for men presenting with infertility and risk factors for testicular cancer, such as cryptorchidism or a previous testicular neoplasm, but not as a routine screening procedure.

**Specialized Clinical Tests on Semen and Sperm**

In some cases, semen analyses have failed to predict fertility accurately, spurring a search for other methods that might improve the diagnostic evaluation of the infertile man. Generally, such specialized clinical tests should be reserved for circumstances where results would clearly help to direct treatment.

**Quantification of Leukocytes in Semen**

Increased numbers of white blood cells in semen have been associated with deficiencies in sperm function and motility. Under wet-mount microscopy, leukocytes and immature germ cells appear quite similar and are properly called “round cells.” Unfortunately, many laboratories improperly report all round cells as “white blood cells,” and in men with such findings, the clinician must ensure that the two types of cells are differentiated. A number of methods are available to distinguish leukocytes from immature germ cells, including traditional cytologic staining and immunohistochemical techniques [22]. Men with true pyospermia (>1 million leukocytes/mL) should be specifically evaluated to exclude genital tract infection or inflammation.

**Tests for Antisperm Antibodies**

Antisperm antibodies (ASA) are a rare cause of male subfertility that do not require routine testing and are typically managed with the use of ICSI. Testing for ASA has historically been done when the semen analysis reveals isolated asthenospermia (with normal sperm concentration) or sperm agglutination. ASAs can be found in the semen, in the seminal plasma, or bound directly to sperm. ASAs can form when the sperm is exposed to large quantities of sperm antigens or after vasectomy. Risk factors for ASA formation include trauma, torsion, biopsy, orchitis, testicular cancer, and vasectomy. Whereas indirect antibody agglutination assays are used to detect ASA in serum or seminal plasma, a direct immunobead test is used to detect ASA (IgG and IgA) bound to the sperm head or tail. Sperm-bound antibodies are thought to be clinically important because they can decrease motility, block penetration of the cervical mucus, and prevent fertilization, thereby decreasing the likelihood for conception [23]. Although some have suggested ASA testing for couples with unexplained infertility, the clinical utility of the test in such couples is uncertain, and ASA testing is unnecessary if ICSI is planned [24]. One recent study has suggested that detection of serum ASA correlates with the presence of spermatogenesis in men with azoospermia and can obviate the need for diagnostic testicular biopsy to help determine whether obstruction is present [25]. Men with azoospermia and ASA are likely to have reproductive tract obstruction. Otherwise, routine testing for ASA is not indicated.

**Sperm Viability Tests**

Sperm viability can be assessed by mixing fresh semen with a supravital dye, such as eosin Y or trypan blue, or by the use of the hypoosmotic swelling (HOS) test [10]. These assays determine whether nonmotile sperm are viable by identifying which sperm have intact cell membranes. In dye tests, viable sperm actively exclude the dye and remain colorless whereas nonviable sperm readily take up the stain. Unfortunately, sperm judged to be viable by means of dye tests can not be used for IVF. In the HOS test, viable nonmotile sperm, which swell when incubated in a hypoosmotic solution, can be used successfully for ICSI [26]. Viable nonmotile sperm can also be identified by means of incubation in pentoxifylline. Viable sperm will develop motility after exposure to pentoxifylline [27].

**Sperm Deoxyribonucleic Acid (DNA) Fragmentation Tests**

DNA integrity is important for normal embryo development. Sperm DNA integrity is maintained in part by the effect of disulfide cross-links between protamines that allow for the compaction of chromatin in the nucleus. Sperm DNA damage can occur as a result of intrinsic factors, such as protamine deficiency and mutations affecting DNA compaction, or from extrinsic factors, such as heat, radiation, and gonadotoxins. The term “DNA fragmentation” refers to denatured or damaged sperm DNA that can not be repaired. A number of clinical tests have been developed to measure sperm DNA fragmentation rates. Direct methods, such as the single-cell gel electrophoresis assay (Comet) and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assays, specifically analyze the number of breaks in the DNA. Indirect tests, such as the sperm chromatin structure assay (SCSA), define abnormal chromatin structure as an increased susceptibility of sperm DNA to acid-induced denaturation in situ [28]. Threshold values used to define an abnormal test are ≥ 25%–27% for the SCSA [29] and ≥ 36% for TUNEL assays [30].

Sperm DNA damage is more common in infertile men and may contribute to poor reproductive performance in some couples. Sperm DNA damage is also associated with spontaneous recurrent miscarriage. However, existing data relating to the relationship between abnormal DNA integrity and reproductive outcomes are too limited to routinely
recommend any of these tests for the male partner in an infertile couple, but the effect of abnormal sperm DNA fragmentation on the value of IUI or IVF and ICSI results may be clinically informative [31]. Although no treatment for abnormal DNA integrity has been proven to have clinical value, varicocele repair and antioxidant use may affect sperm DNA integrity. Sperm retrieved from the tests tend to have better sperm DNA quality in men with abnormal ejaculated sperm DNA integrity [32]. Because the prognostic clinical value of DNA integrity testing may not affect the treatment of couples, the routine use of DNA integrity tests in the clinical evaluation of male-factor infertility is controversial [33].

Less Commonly Used Specialized Tests

Numerous other tests of sperm function have been used predominantly in research studies. Sperm penetration assays may detect defects in sperm fertilizing capacity and could identify patients who would benefit from application of ICSI. However, because ICSI is routinely used during IVF for male-factor infertility couples, this test is rarely of any clinical value. The acrosome reaction of human sperm can be detected with the use of specialized staining techniques. Rates of spontaneous acrosome reactions and acrosome reactions induced by agents such as calcium ionophore and progesterone have been measured. Sperm from infertile men tend to demonstrate higher acrosome levels spontaneously but lower levels in the presence of inducers [34]. A number of biochemical tests of sperm function have been studied, including measurements of sperm creatine kinase [35] and reactive oxygen species (ROS). ROS appear to be generated by both seminal leukocytes and sperm cells and can interfere with sperm function by peroxidation of sperm lipid membranes and creation of toxic fatty acid peroxides [36]. Other tests and procedures have been used to select sperm for ICSI and may identify gametes with better quality, including hyaluronic acid binding, membrane maturity testing, apoptotic evaluation, and magnified sperm examination [37]. However, these tests have a very limited role in the evaluation of male infertility because they have limited clinical utility and typically do not affect treatment.

Genetic Screening

Genetic abnormalities can cause infertility by affecting sperm production or sperm transport. Men with nonobstructive azoospermia or severe oligozoospermia (<5 million/mL) are at increased risk for having a genetic abnormality compared to fertile men [38]. The most common genetic abnormalities found in such men are numeric and structural chromosomal aberrations that impair testicular function and Y-chromosome microdeletions that are associated with isolated defects in spermatogenesis. In addition, most men with CBAVD can be assumed to have an abnormality of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. When indicated, efforts to identify genetic causes for infertility can have a major impact on the choice and outcome of treatment.

Cystic fibrosis gene mutations. There is a strong association between CBAVD and mutations of the CFTR gene, which is located on chromosome 7 [39]. Almost all men with clinical cystic fibrosis exhibit CBAVD. Additionally, as many as 80% of men with CBAVD have documented mutations of the CFTR gene. Failure to detect a CFTR abnormality in men with CBAVD does not exclude the presence of a mutation that cannot be identified with currently available methods. Therefore, most men with CBAVD should be assumed to have a CFTR gene mutation unless they have renal anomalies. To determine the risk of conceiving a child affected with cystic fibrosis, it is important to test the female partner of an affected man. Even if the female partner is negative according to currently available testing, the couple remains at some risk because some of the less common mutations may be missed unless the entire gene is sequenced.

The prevalence of CFTR mutations is also increased among men with azoospermia related to congenital bilateral obstruction of the epididymides and those with unilateral vasa agenesis. Consequently, genetic evaluation should be considered for those having either abnormality. Some men presenting with either unilateral or bilateral vasal agenesis and unilateral renal agenesis have the mesonephric duct abnormalities associated with hereditary renal dysplasia, which has an autosomal dominant form of inheritance with incomplete penetrance and variable expression. These patients do not have CFTR mutations and require genetic counseling before IVF [40, 41].

Karyotypic chromosomal abnormalities. The prevalence of chromosomal abnormalities is increased in infertile men and inversely proportional to sperm count; the prevalence is 10%–15% in azoospermic men [42], ~5% in men with severe oligozoospermia (<5 million/mL), and <1% in men with normal sperm concentrations [43]. Sex chromosomal aneuploidy (Klinefelter syndrome; 47,XXY) accounts for about two thirds of all chromosomal abnormalities observed in infertile men [44]. The prevalence of structural autosomal abnormalities, such as inversions and balanced translocations, also is higher in infertile men than in the general population [45]. Rare azoospermic men may be found to have the 46,XX disorder of sexual development resulting from translocation of sex-determining region Y (SRY) to one of their X chromosomes. Couples in which the male partner has a gross karyotypic abnormality are at increased risk for miscarriages and for having children with chromosomal and congenital defects. Therefore, men with nonobstructive azoospermia or severe oligozoospermia should be evaluated with a high-resolution karyotype before using their sperm to perform ICSI.

Y-chromosome microdeletions. Microdeletions of clinically relevant regions of the Y chromosome have been found in 7% of infertile men with severely impaired spermatogenesis, compared with 2% of normal men. However, the percentage of men with Y-chromosome microdeletions increases to 16% in men with azoospermia or severe oligozoospermia [46]. Such microdeletions are too small to be detected by standard karyotyping, but they can be identified with the use of polymerase chain reaction techniques to analyze sequence-tagged sites that have been mapped along the entire length of the Y chromosome.

Most deletions causing azoospermia or oligozoospermia occur in regions of the long arm of the Y chromosome.
Men with nonobstructive azoospermia or severe oligozoospermia (<5 million/mL) are at increased risk for having a definable genetic abnormality and should be offered karyotype and Y-chromosome analysis before performing ICSI with their sperm. Genetic counseling may be offered when a genetic abnormality is suspected in either the male or the female partner and should be provided whenever a genetic abnormality is detected.

CONCLUSION

An initial screening evaluation of the male partner of an infertile couple is indicated when pregnancy has not occurred after 12 months of unprotected intercourse or after 6 months of failure to conceive when the female partner is >35 years old. Earlier evaluation may be warranted when medical history and physical findings indicate or suggest specific male or female infertility risk factors and for men who question their reproductive potential.

A thorough evaluation by a urologist or other specialist in male reproduction, including a complete medical and reproductive history and physical examination, should be performed if the initial screening evaluation reveals an abnormal male reproductive history or demonstrates abnormal semen parameters. Additional tests aimed at defining the cause may be required.

Acknowledgments: This report was developed under the direction of the Practice Committee of the American Society for Reproductive Medicine (ASRM) as a service to its members and other practicing clinicians. Although this document reflects appropriate management of a problem encountered in the practice of reproductive medicine, it is not intended to be the only approved standard of practice or to dictate an exclusive course of treatment. Other plans of management may be appropriate, taking into account the needs of the individual patient, available resources, and institutional or clinical practice limitations. The Practice Committee and the Board of Directors of the American Society for Reproductive Medicine have approved this report.

The following members of the ASRM Practice Committee participated in the development of this document. All Committee members disclosed commercial and financial relationships with manufacturers or distributors of goods or services used to treat patients. Members of the Committee who were found to have conflicts of interest based on the relationships disclosed did not participate in the discussion.

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REFERENCES


