INVITED REVIEW

# An update on the clinical assessment of the infertile male

**Sandro C. Esteves,[I] Ricardo Miyaoka,[I] Ashok Agarwal[II]**

[I] ANDROFERT – Andrology & Human Reproduction Clinic, Campinas, São Paulo, Brazil. [II] Cleveland Clinic, Ohio, United States.

Male infertility is directly or indirectly responsible for 60% of cases involving reproductive-age couples with fertility-related issues. Nevertheless, the evaluation of male infertility is often underestimated or postponed. A coordinated evaluation of the infertile male using standardized procedures improves both diagnostic precision and the results of subsequent management in terms of effectiveness, risk and costs. Recent advances in assisted reproductive techniques (ART) have made it possible to identify and overcome previously untreatable causes of male infertility. To properly utilize the available techniques and improve clinical results, it is of the utmost importance that patients are adequately diagnosed and evaluated. Ideally, this initial assessment should also be affordable and accessible. We describe the main aspects of male infertility evaluation in a practical manner to provide information on the judicious use of available diagnostic tools and to better determine the etiology of the most adequate treatment for the existing condition.

**KEYWORDS:** Infertility; Diagnosis; Male; Semen Analysis; Review.

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E-mail: s.esteves@androfert.com.br

Tel.: 55 19 3295-8877

## INTRODUCTION

Recently, important advances have been achieved in the field of male infertility in both diagnostics and treatment. Genetic tests have made it possible to correctly classify cases of non-obstructive azoospermia (NOA) previously believed to be idiopathic. Microsurgery has increased the success rates of reproductive tract reconstruction and sperm retrieval either from the testicle or the epididymis. With the advent of gamete micromanipulation, previously infertile men with severe oligozoospermia or azoospermia were given the chance to father children of their own.

Infertility is a common scenario at the urologist's office, and approximately 8% of men of reproductive age seek medical attention for infertility problems. Of these, up to 10% present with a reversible cause affecting their fertility potential; varicocele represents 35% of these cases.[1] As such, the male partner must be systematically evaluated in every investigation of an infertile couple.

Because 80% of couples are able to achieve pregnancy within the first year of attempting, a couple should only be diagnosed as infertile after one year of regular sexual activity without using a contraceptive method. Investigation is initiated earlier when risk factors are present, including advanced maternal (>35 years) or paternal age (>45 years), a history of urogenital surgery, cancer, cryptorchidism, varicocele, orchitis, use of gonadotoxins or genital infections, among others.

The urologist is responsible for diagnosing, counseling and treating the underlying cause whenever possible. When there is no specific treatment he/she is still responsible for referring the patient to a specialized assisted reproductive techniques (ART) center or, if the urologist is a member of an ART center's multi-professional team, for extracting the male gamete from the testicle or epididymis.

### How big is the problem?

For healthy young couples, the probability of achieving pregnancy per reproductive cycle is approximately 20 to 25%. The cumulative probabilities of conception are 60% within the first 6 months, 84% within the first year and 92% within the second year of fertility-focused sexual activity.[2]

Infertility is a common clinical problem affecting 13 to 15% of couples worldwide.[3] The prevalence varies throughout different countries, being higher in the underdeveloped nations where limited resources for diagnosis and treatment exist.[4] In the United Kingdom, infertility is believed to affect one in six couples.[5] According to Kamel, it should be regarded as a public health problem, as it affects not only the health care system but also the social environment.[2]

### What are the mechanisms involved in sperm production and ejaculation?

The testicles are responsible for producing the gametes (spermatozoa) within the seminiferous tubules and sexual hormones (testosterone and androstenedione) in the interstitial cells. According to recent data, the duration of spermatogenesis in humans, from the initiation of sperm

production to ejaculation, is no longer than 60 days.[6] The seminal parameters of an individual actually represent the result of influential biological, physical and occupational factors that have been present within the two months prior to semen collection. The hormonal control of sperm production involves the hypothalamus (gonadotropin releasing hormone [GnRH]), the anterior pituitary gland (gonadotropins – follicle-stimulating hormone [FSH] and luteinizing hormone [LH]), and the testicles (testosterone and inhibin). The storage and maturation of spermatozoa occur in the epididymis. The maturation process is, however, only fully completed within the female reproductive tract. The duration of spermatozoa transit throughout the epididymis is approximately 12 days. Both seminal vesicles and the vas deferens have a common embryologic origin. As such, when congenital bilateral absence of vas deferens (CBAVD) is diagnosed, it is often associated with seminal vesicle hypoplasia/agenesia. This aspect is important in terms of the differential diagnosis of azoospermia. In most CBAVD cases, the semen volume is reduced and the semen does not contain fructose. Under normal conditions, spermatozoa are stored in the epididymides. At the time of ejaculation, ductal and epididymal muscle contractions under sympathetic stimulation conduct the spermatozoa towards the prostatic urethra where they join fluids excreted by both the prostate and seminal vesicles to form semen. Periurethral muscle contraction is responsible for expelling the semen out of the urethra. Interference in any of these steps may lead to male infertility, and the pathophysiological mechanism involved is dependent on which organ or regulatory system is afflicted.

## What are the causes of male infertility/subfertility?

Any process that affects sperm production and quality is potentially harmful to male fertility. The major causes for male infertility include varicocele, genital tract obstruction, testicular failure, cryptorchidism, idiopathic, gonadotoxin exposure, genetic conditions, infections, hormonal dysfunction, immunological conditions, ejaculatory/sexual dysfunction, cancer and systemic diseases.

From a group of 2,383 male infertility patients who attended our tertiary center for male reproduction, 48.4% of the individuals had conditions that were potentially correctable by surgical or medical treatment. The other half consisted of candidates for assisted reproduction, particularly ART involving *in vitro* fertilization (IVF) coupled with intracytoplasmic sperm injection (ICSI) (Table 1).

**Table 1 -** Distribution of Diagnostic Categories in a Group of Infertile Men Who Attended a Male Infertility Clinic.

| Category | N | % |
|---|---|---|
| Varicocele | 629 | 26.4 |
| Infectious | 72 | 3.0 |
| Hormonal | 54 | 2.3 |
| Ejaculatory dysfunction | 28 | 1.2 |
| Systemic diseases | 11 | 0.4 |
| Idiopathic | 289 | 12.1 |
| Immunologic | 54 | 2.3 |
| Obstruction | 359 | 15.1 |
| Cancer | 11 | 0.5 |
| Cryptorchidism | 342 | 14.3 |
| Genetic | 189 | 7.9 |
| Testicular failure | 345 | 14.5 |
| TOTAL | 2,383 | 100.0 |

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## What are the signs?

The typical clinical situation is the presentation of an infertile couple within reproductive age who are sexually active and do not use any contraceptive methods but are unable to achieve pregnancy. In general, the female partner is evaluated by a gynecologist who orders a semen analysis for the male. If the semen analysis results are abnormal, then male infertility is suspected.

## What are the possible confounding causes?

In approximately 1% of cases, male infertility may be the clinical presentation of a more serious or potentially fatal disorder such as testicular or brain cancer, medullary spinal cancer, endocrinopathies, genitourinary malformation, systemic disease and genetic syndromes.[7,8] Testicular cancer is about 50 times more common in infertile men.[9] Therefore, it is important for the clinician to keep in mind that infertility may be the initial manifestation of a more severe medical condition.

## How to proceed with the diagnostic evaluation of the male partner?

**Initial workup.** ***Medical history.*** A detailed medical history should be obtained for any factor that may impact fertility potential. Information regarding the following areas should be collected: a) prior fertility, previous diseases during childhood and puberty such as viral orchitis and cryptorchidism; c) surgeries performed, especially those involving the pelvic and inguinal regions and genitalia; d) genital traumas; e) infections such as orchiepididymitis and urethritis; f) physical and sexual development; g) social and sexual habits; h) exposure to gonadotoxic agents such as radiotherapy or chemotherapy, recent fevers or heat exposures, and i) current or recent medications and j) a family history of birth defects, mental retardation, reproductive failure or cystic fibrosis. In Table 2 we present a summary of the factors that should be considered when assessing the infertile male.

***Physical Examination.*** Appropriate sexual development must be assessed. In the presence of diminished Body hair distribution, gynecomastia or eunuchoid proportions, androgen deficiency should be suspected.

Genital examination can reveal the presence of a hypospadiac urethral meatus, pathologic curvature of the phallus or an active sexually transmitted disease.

Normal adult testicles should have a 4 cm length and a 2.5 cm width, resulting in a volume of approximately 20 mL. Testicular volume can be estimated using a pachymeter or an orchidometer (Figure 1A). Testicles should present with a firm consistency. Approximately 85% of the testicular parenchyma is involved in spermatogenesis, but there is no lower limit for testicular volume to exclude the presence of spermatozoa. As such, testicle size cannot be relied on as a clinical marker to preclude a trial of sperm retrieval.[10]

Both primary and secondary testicular failure may lead to bilateral testicular hypotrophy. When serum testosterone is low, the volume of seminal fluid is often small. An endocrine workup helps to distinguish both conditions. High FSH levels accompanied by normal or low testosterone levels imply primary testicular failure. These patients

**Table 2 -** Clinical Male Infertility History Outline.

| |
|---|
| **1) Infertility History** |
| Age of partners, length of time the couple has been attempting to conceive |
| Contraceptive methods/duration |
| Previous pregnancy (current partner/other partner) |
| Previous treatments |
| Treatments/evaluations of female partner |
| **2) Sexual History** |
| Potency, libido, lubricant use |
| Ejaculation, timed intercourse, frequency of masturbation |
| **3) Childhood and Development** |
| Cryptorchidism, hernia, testicular trauma |
| Testicular torsion, infection (e.g., mumps) |
| Sexual development, puberty onset |
| **4) Personal History** |
| Systemic diseases (diabetes, cirrhosis, hypertension) |
| Sexually transmitted diseases, tuberculosis, viral infections |
| **5) Previous Surgeries** |
| Orchidopexy, herniorrhaphy, orchiectomy (testicular cancer, torsion) |
| Retroperitoneal and pelvic surgery |
| Other inguinal, scrotal and perineal surgery |
| Bariatric surgery, bladder neck surgery, transurethral resection of the prostate |
| **6) Gonadotoxin Exposure** |
| Pesticides, alcohol, cocaine, marijuana abuse |
| Medication (chemotherapy agents, cimetidine, sulfasalazine, nitrofurantoin, allopurinol, colchicine, thiazide, β– and α–blockers, calcium blockers, finasteride) |
| Organic solvents, heavy metals |
| Anabolic steroids, tobacco use |
| High temperatures, electromagnetic energy |
| Radiation (therapeutic, nuclear power plant workers), etc. |
| **7) Family History** |
| Cystic fibrosis, endocrine diseases |
| Infertility in the family |
| **8) Current Health Status** |
| Respiratory infection, anosmia |
| Galactorrhea, visual disturbances |
| Obesity |

should be offered a genetic evaluation for chromosomal abnormalities and Y chromosome microdeletions. The combination of low serum FSH levels and low testosterone levels suggests hypogonadotropic hypogonadism, especially if bilateral atrophic testicles are present. In this scenario, serum LH is also often low. These men should undergo cranial imaging and ''serum prolactin assessment'' to exclude pituitary gland diseases.[11]

The epididymides should also be evaluated according to their size and consistency. A healthy epididymis should be firm, but an obstructed epididymis is augmented and ingurgitated (soft). The partial or complete absence regression of an epididymis may represent CBAVD. The vasa are easily palpable inside the posterior aspect of the spermatic cord as a distinct, firm, round, ''spaghetti-like'' structure. A unilateral or bilateral congenital absence of the vas results in oligozoospermia or azoospermia, respectively. Narrowing areas of the vasa deferentia may represent a sequelae resulting from an infection or trauma.

The absence of the vasa deferentia or vasal agenesis is a clinical diagnosis and does not depend on complementary imaging. However, 25% of men with unilateral vasal agenesis and approximately 10% of men with CBAVD also have unilateral renal agenesis and should undergo an abdominal ultrasound to identify this condition.[12]

Physical examination is the method of choice for the diagnosis of varicocele and should be performed with the patient standing in a warm room[13]. This method has a sensitivity and specificity of approximately 70%.[14] Varicoceles diagnosed by this method are termed ''clinical'' and may be graded according to their size. In varicocele patients, a venous dilation exists and may be enhanced during the Valsalva maneuver. Large visible veins seen through the scrotal skin are indicative of grade III varicoceles (Figure 2). Moderate (grade II) and small-sized varicoceles (grade I) have dilated veins that are palpable without and with the aid of the Valsalva maneuver, respectively.[15] No standardized diagnostic method has been defined for the identification of varicoceles.[16]

Inguinal and genital areas may reveal scars from previous surgical interventions such as hydrocele correction and inguinal hernia repairs which may account for damage to the testicular blood supply and to the vas deferens.

**Semen analysis.** Although semen analysis does not test sperm function, it is the cornerstone of the initial laboratory

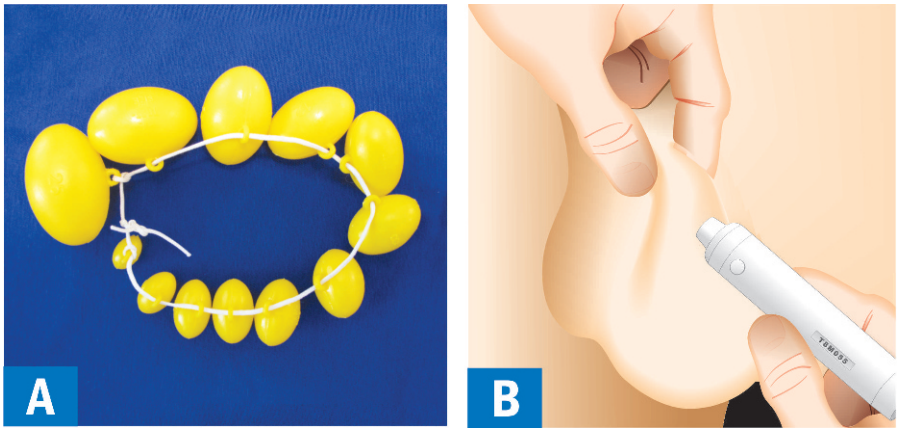



**Figure 1 -** Tools commonly used during the physical examination of subfertile males. A) Photograph of the Prader orchidometer. It is used to measure the volume of the testicles and consists of a chain of 12 numbered beads of increasing size from 1 to 25 mL. The beads are compared with the testicles of the patient and the volume is read off the bead that most closely matches the size of the testicle. Prepubertal sizes are 1 to 3 mL, pubertal sizes are 4 to 12 mL and adult sizes are 15 to 25 mL. B) Schematic illustration depicting the use of the 9 Mhz pencil-probe Doppler stethoscope for varicocele examination. The patient is examined in the upright position and the conducting gel is applied to the upper aspect of the scrotum. A venous ''rush'' may be heard during the Valsalva maneuver, indicating blood reflux.

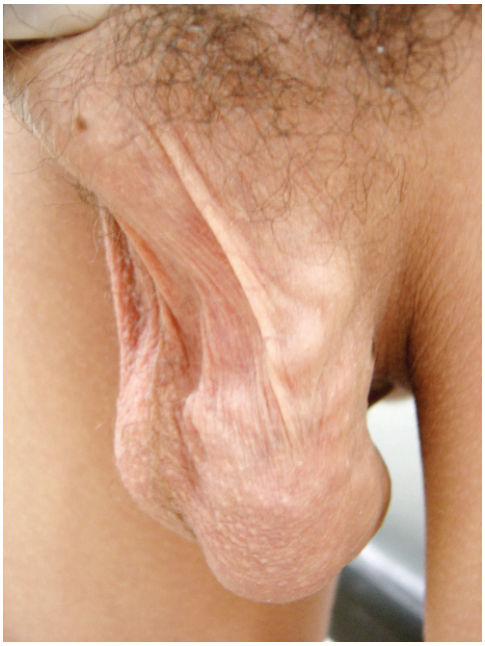


**Figure 2 -** Photograph of a large left grade III varicocele that can be seen through the scrotal skin.

evaluation and provides information on the status of the germ epithelium, epididymides and the accessory sexual glands. The analysis may also provide data from which a prognosis of fertility or the diagnosis of infertility can be extrapolated.[17]

It is recommended that the semen is collected in a specialized andrology laboratory and analyzed by well-trained technicians under rigorous quality control standards.[18] Nonetheless, the prognostic value of semen components, such as sperm number, motility and morphology, as surrogate markers of male fertility is influenced by many other factors related to sexual activity and the function of accessory sex glands, among others. Routine semen analysis has its own limitations and does not account for putative sperm dysfunctions such as immature chromatin or fragmented DNA. Results from at least two, but preferably three, separate seminal analyses must be obtained before a definitive conclusion can be drawn, as wide biological variability may exist within the very same individual. The interval between the analyses is arbitrary and is generally recommended to be 1-2 weeks. Ejaculatory abstinence should occur for a minimum of two days to a maximum of seven days, with two to three days being ideal.[19] Longer periods of abstinence may lead to higher ejaculatory volumes and increased spermatozoa quantity, but motility is usually negatively affected. The specimen is collected by masturbation, and care must be taken to avoid spillage outside the container which can be misinterpreted as hypospermia. Collection should preferentially be done in a private room and no lubricant should be used. If collected at home, the specimen should be brought to the lab within 30 minutes and should be kept close to the body in an effort to maintain physiological temperature during transportation. The specimen must be allowed to liquefy for 30 to 60 minutes before being analyzed. Routine seminal analysis should include a) the physical characteristics of semen, including liquefaction, viscosity, pH, color and odor; b) specimen volume; c) sperm count; d) sperm motility and progression; e) sperm morphology; f) leukocyte quantification and g) fructose detection in cases where no spermatozoa are found, especially if the total volume of the sample is less than 1 mL. The World Health Organization (WHO) criteria used to define normality have recently been updated,[19] as shown in Table 3. Approximately 2,000 men from eight countries whose partners had a time-to-pregnancy of $\leq$ 12 months were chosen to provide reference distributions for semen parameters. One-sided lower reference limits (the fifth centile) were generated and have been proposed to be used as the lower cutoff limits for normality (Table 3). Apart from the total sperm number per ejaculate, the lower limits of these distributions are lower than the previously presented normal or reference values[20-22] but are in agreement with recent observations made from studying populations of fertile and infertile couples.[23-25]

A morphometric description of spermatozoa using the strict criteria described by Kruger[26] was incorporated in the new WHO guidelines. Low values of 3–5% normal forms have been found by others to be optimal cut-off values between fertile and infertile men whose spermatozoa were used for in vitro fertilization,[27] intrauterine insemination[28] and in spontaneous pregnancies.[29] Interpretation of the reference ranges requires an understanding that they provide a description of the semen characteristics of recent

**Table 3 -** Reference Values for Semen Parameters, as Published in Consecutive WHO Manuals.

| Semen parameters | WHO, 1992 | WHO, 1999 | WHO, 2010[1] |
|---|---|---|---|
| *Volume* | $\geq$ 2 mL | $\geq$ 2 mL | 1.5 mL |
| *Sperm concentration/mL* | $\geq 20 \times 10^6$/mL | $\geq 20 \times 10^6$/mL | $15 \times 10^6$/mL |
| *Total sperm concentration* | $\geq 40 \times 10^6$ | $\geq 40 \times 10^6$ | $39 \times 10^6$ |
| *Total motility (% motile)* | $\geq$ 50% | $\geq$ 50% | 40% |
| *Progressive Motility*[2] | $\geq$ 25% (grade a) | $\geq$ 25% (grade a) | 32% (a + b) |
| *Vitality (% alive)* | $\geq$ 75% | $\geq$ 75% | 58% |
| *Morphology* | $\geq$ 30%[3] | 14%[4] | 4%[5] |
| *Leukocyte count* | $< 1.0 \times 10^6$/mL | $< 1.0 \times 10^6$/mL | $< 1.0 \times 10^6$/mL |

WHO = World Health Organization.
[1]Lower reference limit obtained from the lower fifth centile value.
[2]Grade a = rapid progressive motility ($>$ 25 μm/s); grade b = slow/sluggish progressive motility (5–25 μm/s); Normal = 50% motility (grades a + b) or 25% rapid progressive motility (grade a) within 60 min of ejaculation.
[3]Arbitrary value.
[4]No actual value given, but multicenter studies refer to $>$ 14% (strict criteria) for *in vitro* fertilization (IVF).
[5]Normal shaped spermatozoa according to Tygerberg (Kruger) strict criteria.

fathers. The reference limits should not be over-interpreted to distinguish fertile men from infertile men, although they represent the semen characteristics associated with couples who have achieved pregnancy within 12 months of unprotected sexual intercourse. No single aspect of semen analysis was able to solely distinguish between fertile and infertile men, although morphology was suggested to be the most important. The coexistence of multiple altered seminal parameters increases the risk for infertility,[23] but semen characteristics need to be interpreted in conjunction with the patient's clinical information. The reference limits provided by the WHO manual are from semen samples that initiated natural conceptions and help in identifying men who may need infertility treatment, but they should not be used to determine the nature of that treatment.

Abnormal white blood cell (leukocyte) counts are a frequent cause of male infertility. The incidence of leukocytospermia (>1 million leukocytes/mL of semen) in infertile men varies between 3 and 23% and has been correlated with clinical and subclinical genital infections, elevated levels of reactive oxygen species, elevated antisperm antibody levels and deficient sperm function.[30] Neutrophils are predominant among the inflammatory cells and may be both identified and quantified using different methods and coloring techniques such as as peroxidase, morphology, granulocyte elastase and immunohistology. The Endtz test is a simple low-cost option to detect the presence of peroxidase within neutrophils,[31] and it has been widely used to determine the presence of granulocytes in the semen.

Regarding sperm counts, azoospermic patients should have their diagnosis confirmed by verifying the lack of any spermatozoa in centrifuged seminal fluid on two separate occasions using a high-powered microscope. Azoospermia with low ejaculate volume (<1.0 mL) not related to hypogonadism or CBAVD can be caused by ejaculatory dysfunction, although the most common cause is ejaculatory duct obstruction (EDO). When suspected, EDO can be confirmed by assessing the seminal pH and fructose levels, as normal semen is alkaline and contains fructose.

**Complementary workup.** ***Endocrine evaluation.*** Endocrine evaluation is suggested when the following scenarios are present[32]: a) a sperm concentration < 10 million/mL; b) erectile dysfunction; c) hypospermia (volume <1 mL) or d) signs and symptoms of endocrinopathies or hypogonadism. The minimal evaluation includes the assessment of serum follicle-stimulating hormone (FSH) and testosterone levels, which reflect germ cell epithelium and Leydig cell status, respectively. If the testosterone level is low, a second collection is recommended along with free testosterone, LH and prolactin measurements. Isolated FSH elevation is usually indicative of severe germ cell epithelium damage. Highly elevated FSH and LH levels, when associated with low-normal or below normal testosterone levels, suggest diffuse testicular failure and may have either a congenital (e.g., Klinefelter syndrome) or acquired cause. Concomitant low levels of FSH and LH may implicate hypogonadotropic hypogonadism. This condition may be congenital or secondary to a prolactin-producing pituitary tumor.

Gonadotropin values within the normal range suggest an extraductal obstruction in azoospermic subjects. However, azoospermic patients with testicular failure and testis histology exhibiting sperm maturation arrest and 10% of those diagnosed with Sertoli-cell-only syndrome may present with non-elevated FSH levels.[32]

Serum estradiol levels should be determined in patients presenting with gynecomastia. Infertile patients with a testosterone to estradiol ratio less than 10 can harbor significant but reversible seminal alterations.[33] Vaucher et al.[34] suggested that hyperestrogenism secondary to a higher conversion rate of testosterone into estradiol in Klinefelter syndrome (KS) patients inhibits testosterone production via a negative feedback pathway and may indicate the overexpression of aromatase CYP19 in the testis. As such, there would be a scientific rationale for the use of aromatase inhibitors in KS patients.[33]

In azoospermic men with a normal ejaculate volume, FSH serum level greater than two times the upper limit of the normal range is reliably diagnostic of dysfunctional spermatogenesis and, when found, a diagnostic testicular biopsy is usually unnecessary, although no consensus exists in this matter.[11]

Serum prolactin levels should be determined in infertile men with a complaint of concomitant sexual dysfunction or when there is clinical or laboratory evidence of pituitary disease; however, hyperprolactinemia is rarely a cause of infertility in healthy men[35]. Although hormonal alterations may be present in approximately 10% of men who undergo assessment, clinically significant changes affect less than 3% (Table 1).

***Genetic evaluation.*** Male infertility can be associated with various genetic factors, including chromosomal aberrations, genetic alterations and Y chromosome microdeletions. Chromosomal aberrations are assessed through G-band karyotyping. Genetic mutations and Y chromosome microdeletions may be also assessed by the analysis of the peripheral blood as such, DNA is amplified by the polymerase chain reaction (PCR) biomolecular technique. Table 4 summarizes the indications and recommended tests for genetic evaluations.

Chromosomal abnormalities may be present in approximately 6% of infertile men, and the prevalence is inversely correlated with sperm count. Azoospermic men can be affected in up to 16% of cases.[36] Sex chromosomal aneuploidy (Klinefelter syndrome; 47,XXY) is the most

**Table 4 -** Indications for Genetic Testing in Male Infertility.

| Indications | Recommended tests |
|---|---|
| Men with infertility of unknown etiology and sperm concentrations < 10 million/mL who are candidates for ART | Y chromosome microdeletion and G-band karyotyping |
| Non-obstructive azoospermia in a male considering testicular sperm retrieval for ART | Y chromosome microdeletion and G-band karyotyping |
| Azoospermic or oligozoospermic men with the absence of at least one vas deferens at physical examination | CFTR gene mutation analysis |
| Azoospermic men with signs of normal spermatogenesis (e.g., obstructive azoospermia of unknown origin) | CFTR gene mutation analysis |
| History of recurrent miscarriage or personal/familiar history of genetic syndromes | G-band karyotyping |

ART = assisted reproductive techniques.
G-band karyotyping = Giemsa band karyotyping.
CFTR = cystic fibrosis transmembrane conductance regulator.

frequent chromosomal disorder present in infertile men and is generally associated with hypotrophic or atrophic testicles, elevated serum FSH levels and azoospermia, although spermatogenesis can be differently affected in patients with a mosaic karyotype (46,XY/47,XXY). The mutation of the cystic fibrosis gene (CFTR gene), which is located on the long arm of chromosome 7, is also a relatively common genetic disorder. Depending on the mutation length, cystic fibrosis can manifest as its full clinical presentation (an autosomic recessive potentially fatal disease) or as a mild form that affects approximately 1.3% of infertile men in which there is a congenital bilateral absence of the vasa deferentia. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations compromise the development of Wolffian duct-derived structures (efferent ducts, epididymis and vasa deferentia) and may also be implicated in seminal vesicle hypoplasia or agenesis and unilateral renal agenesis. Approximately 80% of men presenting with CBAVD have a CFTR mutation, and as the genetic testing is not 100% sensitive, these subjects should be assumed to harbor the genetic anomaly. Testing should also be offered to the female partner, as she may also be a carrier (an approximately 4% risk), before using his sperm for assisted conception. After genetic testing, genetic counseling should be offered.[11] Recent data suggest that azoospermic men with an idiopathic obstruction and those presenting with the triad composed by chronic sinusitis, bronchiectasis and obstructive azoospermia (Young Syndrome) have an elevated risk for the CFTR mutation.[36] The long and short arms of the Y chromosome are related to spermatogenesis and testicle development, respectively. The Y chromosome region related to infertility is called the azoospermia factor (AZF) locus. The locus can harbor complete or partial microscopic deletions that are isolated or in combination with one another, and in non-overlapping subregions called AZFa, AZFb, AZFc and AZFd. These subregions contain multiple genes that control different steps of spermatogenesis.

The most common Y chromosome deletion in infertile men is the one affecting the DAZ gene (deleted in azoospermia) located in the AZFc region. Severe oligozoospermia or azoospermia can be seen in these cases. Y chromosome microdeletions are found in 15% of men with azoospermia and in 6% of men presenting with severe oligozoospermia ($<$1 million/mL).[36] For sperm counts between 1 and 5 million/mL, the detection rate drops down to 1.7%.[37] Detecting Y chromosomal microdeletions provides predictive information regarding the success of sperm retrieval for intracytoplasmic sperm injection (ICSI). AZFa and AZFb microdeletions present as azoospermia are associated with germ cell aplasia and maturation arrest, respectively. In such cases, attempts at sperm retrieval are not recommended because there is virtually no chance of finding testicular sperm.[38,39] In AZFc microdeletion cases, sperm can be retrieved in approximately 71% of patients.[37] Clinical pregnancy rates obtained via assisted conception are essentially the same for idiopathic azoospermic patients who can also have their sperm retrieved.

Testing also yields information for genetic counseling, as sons of men with Y chromosomal microdeletions will inevitably inherit the abnormality and may also be infertile.[40,41]

Structural abnormalities in the autosomes, such as inversions and translocations, are also higher in infertile men. Gross karyotypic abnormalities are related to an elevated risk of miscarriages and having children with both chromosomal and congenital defects. As such, men with non-obstructive azoospermia or severe oligozoospermia should be karyotyped before their sperm are used for ICSI.[11]

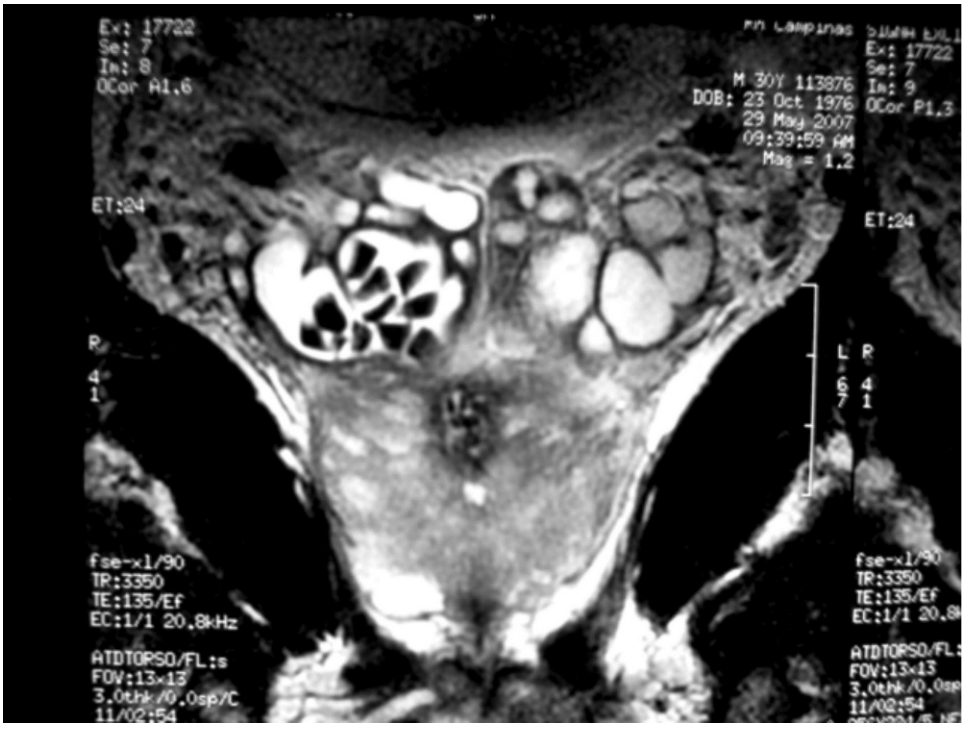



**Figure 3 -** Magnetic resonance imaging showing enlarged seminal vesicles with lithiasis.

***Transrectal, scrotal and renal ultrasonography.*** Transrectal ultrasonography (TRUS) is recommended in certain situations, including low semen volumes (<1.5 mL), abnormal digital rectal examination (DRE) and ejaculatory disorders (anejaculation, hematospermia, painful ejaculation).

TRUS allows for the evaluation of the distal extraductal system (seminal vesicles and ejaculatory ducts). EDO may be congenital or acquired and may present as either complete or partial. This disorder can be identified with TRUS by the presence of seminal vesicle enlargement and by the visualization of cysts at the ejaculatory ducts.[42] When CBAVD is diagnosed, TRUS can reveal abnormalities such as hypoplasia or agenesis at the seminal vesicles. A recent study has suggested that a combination of scrotal ultrasound and TRUS may not only distinguish between obstructive and non-obstructive azoospermia (NOA) but may also determine the etiologic classification of obstructive azoospermia (OA). The sensitivity, specificity and accuracy of combined assessment in discriminating between OA and NOA were 95.3%, 97.2% and 96.0%, respectively.[43] Seminal vesicle aspiration and seminal vesiculography may be performed under TRUS guidance and may help to establish the diagnosis of EDO.[44]

Scrotal ultrasonography should be performed to evaluate palpable nodules or testicular masses. Its use for subclinical varicocele diagnosis is controversial, as several studies have demonstrated that there is no clinical benefit from surgical treatment in this situation.[45] When there is a doubtful physical examination, such as in obese patients, or there is difficulty in assessing the contralateral side of a clinically detectable varicocele, then ultrasonography is useful; correction of a subclinical varicocele concomitant to a clinical contralateral varicocele might be justified in this scenario. The commonly accepted color Doppler ultrasonography criterion for the diagnosis of a varicocele (maximum vein diameter of 3 mm or greater) has a sensitivity of about 50% and a specificity of 90% compared with 70% of physical examination.[14, 46] A pencil-probe Doppler (9 MHz) stethoscope is an inexpensive tool that may aid in the diagnosis of the varicocele. The patient is examined in the upright position, and a venous ''rush'' caused by blood reflux is heard during the performance of the Valsalva maneuver (Figure 1B). However, Hirsh et al. demonstrated that more than 50% of men without clinical varicoceles exhibited a Valsalva maneuver Doppler-positive reflux[47], and therefore this data should be interpreted in conjunction with other patient's findings.

Unfortunately, none of these adjunctive diagnostic methods can precisely differentiate between clinical and subclinical varicoceles, and the significance of a positive test result using any of these adjuvant techniques in infertile men remains uncertain.

Urinary tract ultrasonography is suggested for the evaluation of renal status in patients diagnosed with CBAVD. Renal agenesis may be present in 10% of patients with CBAVD and 25% of those with a unilateral absence of the vas deferens.[48]

***Magnetic Resonance Imaging.*** The use of magnetic resonance imaging (MRI) in infertility investigations has gained importance in recent years. Situations such as the presence of a varicocele, EDO, seminal vesicle agenesis and undescended testis can be seen.[49]

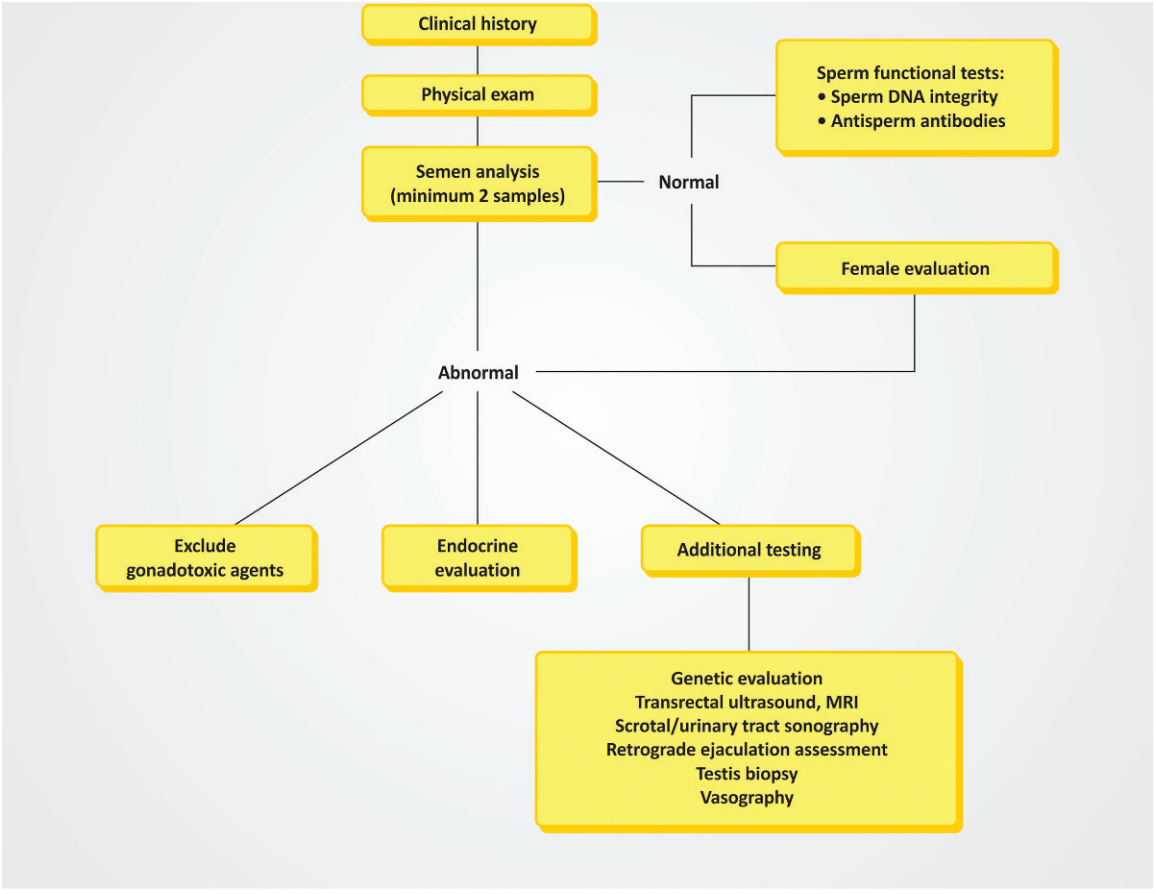
Pelvic MRI helps to clarify, in detail, the pictorial changes initially seen with TRUS (Figure 3). Moreover, MRI has traditionally been used to exclude cranial pathologies manifested by hormonal disturbances.

There is evidence of the optimal usefulness of pituitary MRI in men with hypogonadism when prolactin levels are greater than twice the normal range or have symptoms suggesting a worrisome intracranial abnormality (such as headache, visual disturbances or diffuse metabolic derangements, among others).[50] In general, pituitary abnormalities can be identified in 25% of hypogonadal men. Of these, however, empty sella and pituitary non-functional microadenomas do not require a specific treatment and cast doubts about the cost-effectiveness of their diagnosis.

Nuclear magnetic resonance spectroscopy has recently been proposed as a possible tool to identify metabolic signatures for different histological states in infertile men. On the basis of the ex vivo analysis of testicular biopsy specimens, concentrations of 19 tissue metabolites were acquired and then reassessed in men with a diagnosis of NOA. A singular pattern could be determined for two testis histological states: normal and Sertoli-cell-only (SCO) Syndrome. Proliferating germ cells are linked to high phospholipid synthesis and elevated phosphocholine levels. A normal spermatogenesis spectroscopic pattern shows high peaks of phosphocholine, unlike in cases of SCO. Further research in this area may aid in the identification of a distinct metabolic signature for the presence of sperm, regardless of testis histopathology.[51]

***Sperm Function Laboratory Tests.*** In 10 to 20% of infertile couples who undergo basic infertility investigations, all diagnostic workups will yield normal results, and the couples will be etiologically classified as having idiopathic infertility. Additional tests have been developed to identify functional disorders and other sperm abnormalities which are not addressed by conventional semen analysis. Some of the additional tests are only utilized as research tools.[52] Others, such as sperm DNA fragmentation and anti-sperm antibody testing, have already been implemented in clinical practice.

Sperm DNA fragmentation seems to be one of the most important causes of reduced fertility potential[9]. Sperm populations with decreased DNA integrity is often associated with advanced paternal age, inadequate dietary intake, drug abuse, environmental pesticide exposure, tobacco use, varicocele, medical diseases, hyperthermia, air pollution, genital inflammation and infectious diseases. Fragmentation can be secondary to internal factors such as apoptosis and oxidative stress (a physiological mechanism that is secondary to a high concentration of free radicals), or external factors such as the presence of leukocytes. An assessment of sperm DNA integrity is suggested in the following situations: a) infertile men present with a normal semen analysis as determined by conventional methods; b) recurrent spontaneous abortion; c) to determine the most suitable assisted reproductive technology. Abnormally fragmented sperm DNA can be found in 5% of infertile men with normal semen analyses and in 25% of infertile men with abnormal parameters, but is rarely seen in fertile men.[9] The most common methods used to determine fragmentation rates are Transferase-mediated dTUP nick-end labeling (TUNEL), Comet, the acridine orange test and the SCSA (Sperm Chromatin Structure Assay). The TUNEL technique seems to be the most suitable test, as it offers the possibility to precisely identify all existing endogenous breaks in sperm DNA. This test combines both enzymatic



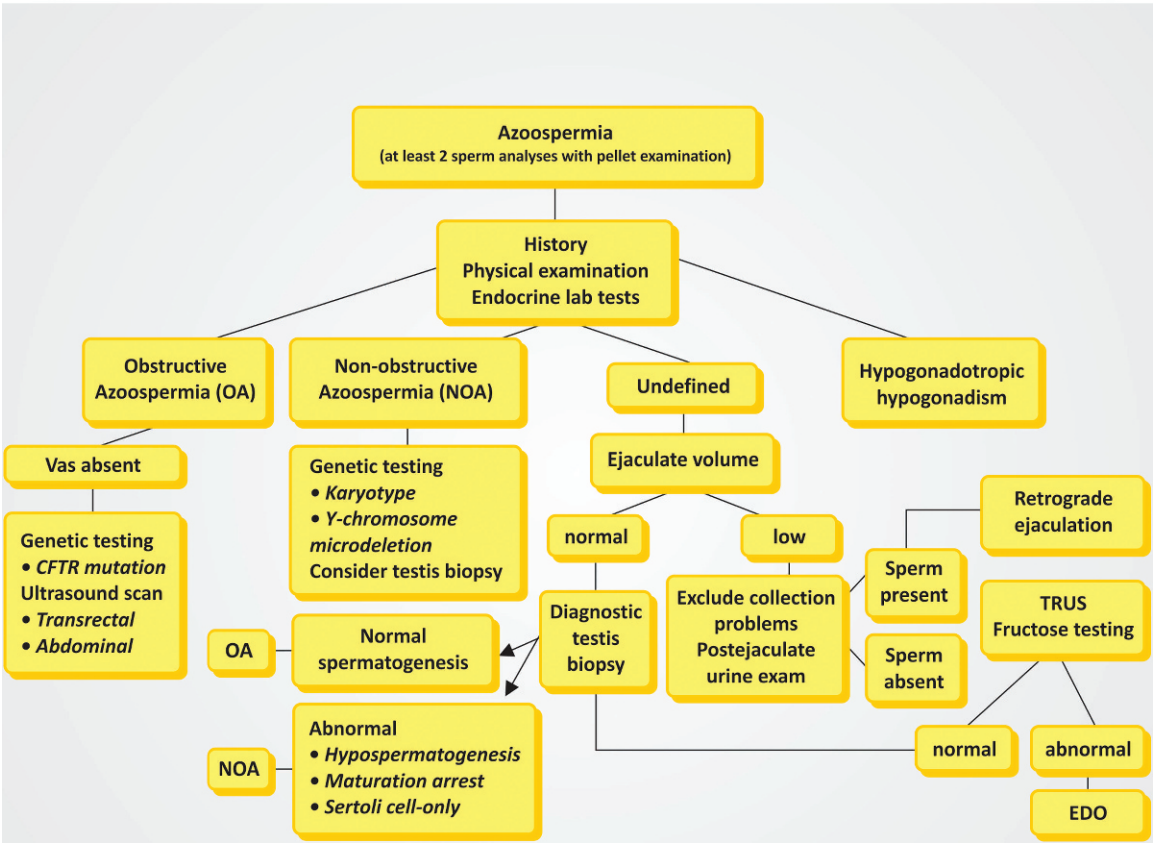




**Figure 4 -** Algorithms for the workup of the infertile male. Algorithm to be considered on initial assessment (top). Algorithm for the management of the patient presenting with azoospermia (bottom).

and immunohistochemical techniques for the direct observation of DNA fragmentation using fluorescence microscopy or flow cytometry. Elevated sperm DNA fragmentation rates significantly diminish the chances for natural and assisted pregnancies.[53]

Risk factors for anti-sperm antibody (ASA) formation include genital infections, testicular trauma or surgical biopsy, cryptorchidism, testicular damage secondary to excessive heat exposure and obstruction of the extratesticular ductal system (e.g., vasectomy). ASA can alter sperm motility and sperm-oocyte interaction. The immunobead test uses human anti-immunoglobulin antibody-coated polyacrylamide beads that are capable of detecting IgA and IgG, the two most clinically important immunoglobulin subtypes associated with immunological infertility.[54] This assay precisely detects antibodies in the serum, in seminal plasma or bound to the surface of the sperm. Rates of sperm binding to beads above 20% are considered clinically significant. Antibodies that are bound to sperm are the most likely to induce functional alterations, such as dysfunctions in motility, vitality and the capability of the sperm to interact with eggs.

Advances in biomolecular techniques are transforming the scientific landscape of sperm cell biology. Novel tests are emerging from andrology research laboratories that may soon become clinically available. The 'omics revolution, as it is termed, refers to the study of genes (genomics), transcripts (transcriptomics), proteins (proteomics) and the various metabolites (metabolomics).[55] With these technologies, inventories of lipids, proteins, metabolites and RNA species may be determined. The application of 'omics technologies to spermatozoa, in concert with detailed assessments of their functional competence, may provide insights into the biochemical basis of defective semen quality.[56] This information is likely to be useful in understanding the causes of male infertility and in the development of rational methods for its treatment and possible prevention.

***Vasography.*** Vasography is indicated in a few select cases of obstructive azoospermia and when there is reason to suspect a unilateral obstruction (e.g., in the case of a previous surgical intervention) in severe oligozoospermic men with a hypotrophic contralateral testis. Vasography and seminal vesiculography are usually undertaken by scrotal or transrectal routes. The presence of sperm upon microscopic analysis of the fluid recovered from the vas deferens and seminal vesicles indicates a distal obstruction and rules out a testicular or epididymal obstruction.

Currently, vasography is only performed concomitantly with a surgical approach to resolve an obstruction.

**Testis Biopsy.** Testis biopsies are suggested in select cases of azoospermia or severe oligozoospermia to distinguish between obstructive and non-obstructive cases. This distinction is necessary to accurately determine the histopathology of the testis, which may reveal normal spermatogenesis, hypospermatogenesis, germ cell maturation arrest, germ cell aplasia (Sertoli-cell-only syndrome), tubular sclerosis or a combination of these conditions. Biopsies can be performed using percutaneous or open approaches. In cases in which biopsies are obtained for diagnostic purposes only, the authors' technical choice is to perform the procedure either percutaneously or using the ''window'' open technique without testis delivery from the scrotum. Specimens should be placed in a fixative solution such as Bouin's, Zenker's or glutaraldehyde; formalin should not be used as it may disrupt the tissue architecture. In cases of NOA, histology results provide important prognostic information regarding the likelihood of retrieving spermatozoa that can be used in ART[57]. However, as spermatogenesis is often limited and focal in NOA patients, the biopsy should ideally be performed in specialized assisted reproduction centers to allow for sperm cryopreservation and to avoid the need for repeated procedures.

## Final Considerations

Male infertility may be responsible for 40-60% of cases where a couple is unable to conceive and should not be underestimated. A precise and detailed medical history, physical examination, semen analyses and complementary tests, as appropriate, are the key to obtaining a correct diagnosis and to determining the best treatment strategies. Figure 4 depicts algorithms that may aid in the initial assessment of the infertile male.

Semen parameters within the reference interval do not guarantee fertility, nor do values outside those limits necessarily imply male infertility or pathology. They must be interpreted within the context of the patient's clinical information. The reference limits provided by the WHO manual are derived from semen samples used to initiate natural conceptions; they may indicate a need for infertility treatment but not the nature of that treatment.

When no clear cause of infertility can be determined, additional tests such as genetic testing, sperm DNA fragmentation and anti-sperm antibody assessments should be considered. Novel molecular sperm function tests are emerging in the field of andrology. The application of proteomics, transcriptomics and metabolomics to analyze sperm should provide a more comprehensive analysis of the biochemical basis of defective semen quality.

Genetic evaluation is recommended in cases of severe oligozoospermia and azoospermia, as this analysis may aid in identifying cases in which sperm retrieval is actually possible and may also help in counseling couples about their potential offspring.

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