The Optimal Evaluation of the Infertile Male: AUA Best Practice Statement

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Abbreviations and Acronyms

ASA	antisperm antibodies
AUA	American Urological Association
BOD	Board of Directors
CASA	computer-aided sperm analysis
CBAVD	congenital bilateral absence of the vasa deferentia
CFTR	cystic fibrosis transmembrane conductance regulator
CLIA	clinical laboratory improvement amendments
FSH	follicle-stimulating-hormone
HOS	hypoosmotic swelling
ICSI	intracytoplasmic sperm injection
IUI	intrauterine insemination
IVF	in vitro fertilization
LH	luteinizing hormone
PGC	Practice Guidelines Committee
RCTs	randomized controlled trials
ROS	reactive oxygen species
SPA	sperm penetration assay
TRUS	transrectal ultrasonography
WHO	World Health Organization

Introduction

Approximately 15% of couples are unable to conceive after one year of unprotected intercourse. A male factor is solely responsible in about 20% of infertile couples and contributory in another 30-40%.[1] If a male infertility factor is present, it is almost always defined by the finding of an abnormal semen analysis, although other male factors may play a role even when the semen analysis is normal. This review offers recommendations for the optimal diagnostic evaluation of the male partner of an infertile couple. Male infertility can be due to a variety of conditions. Some of these conditions are identifiable and reversible, such as ductal obstruction and hypogonadotropic hypogonadism. Other conditions are identifiable but not reversible, such as bilateral testicular atrophy secondary to viral orchitis. When identification of the etiology of an abnormal semen analysis is not possible, as is the case in many patients, the condition is termed idiopathic. When the reason for infertility is not clear, with a normal semen analysis and partner evaluation, the infertility is termed unexplained. Rarely patients with normal semen analyses have sperm that do not function in a manner necessary for fertility. The purpose of the male evaluation is to identify these conditions when present. Identification and treatment of reversible conditions may improve the male's fertility and allow for conception through intercourse. Even azoospermic patients may have active sperm production or could have sperm production induced with treatment. Detection of conditions for which there is no treatment will spare couples the distress of attempting ineffective therapies. Detection of certain genetic causes of male infertility allows couples to be informed about the potential to transmit genetic abnormalities that may affect the health of offspring. Thus, an appropriate male evaluation may allow the couple to better understand the basis of their infertility and to obtain genetic counseling when appropriate. If specific corrective treatment is not available, it still may be possible to employ assisted

reproductive techniques such as testicular or epididymal sperm retrieval with intracytoplasmic sperm injection (ICSI). Alternatively, such couples may consider therapeutic donor insemination or adoption. Finally, male infertility may occasionally be the presenting manifestation of an underlying life-threatening condition.[2] Failure to identify diseases such as testicular cancer or pituitary tumors may have serious consequences, including, in rare cases, death. The goals of the optimal evaluation of the infertile male are to identify:

- potentially correctable conditions;
- irreversible conditions that are amenable to assisted reproductive techniques using the sperm of the male partner;
- irreversible conditions that are not amenable to the above, and for which donor insemination or adoption are possible options;
- life- or health-threatening conditions that may underlie the infertility and require medical attention; and
- genetic abnormalities that may affect the health of offspring if assisted reproductive techniques are to be employed.

Methodology

This best practice statement, *Optimal Evaluation of the Infertile Male*, is part of an updated series on male infertility prepared by the Male Infertility Best Practice Statement Panel (Appendix 1). Other titles include: *Best Practice Statement on Evaluation of the Azoospermic Male*, *Best Practice Statement on Management of Obstructive Azoospermia* and *Best Practice Statement on Varicocele and Infertility*. The first editions (2001) of these 4 documents were prepared by the Male Infertility Best Practice Policy Committee of the American Urological Association, Inc.® (AUA; Appendix 1) and the Practice Committee of the American Society for

Reproductive Medicine. The two organizations had agreed to collaborate to prepare documents of importance in the field of male infertility.

In October 2007, an updated assessment of the literature on male infertility by the AUA Practice Guidelines Committee (PGC) found insufficient outcomes data to support a formal meta-analysis and an evidence-based guideline. The evidence was generally of a low level, being derived overwhelmingly from nonrandomized studies. Thus, the Male Infertility Best Practice Statement Panel, which included many of the members of the 2001 Committee, was created by the Board of Directors (BOD) of the AUA. The Panel was charged with developing a best practice statement, based on the previous report, by employing published data in concert with expert opinion. The Panel co-chairmen and members were selected by the PGC. The mission of the Panel was to develop recommendations, based on expert opinion, for optimal clinical practices in the diagnosis and treatment of male infertility. It was not the intention of the Panel to produce a comprehensive treatise on male infertility.

The Medline search spanning 1999 through October 2007 was supplemented by review of bibliographies and additional focused searches. In all, 341 articles were deemed by the Panel members to be suitable for scrutiny. Three of the four original 2001 reports were updated with new findings and are presented in the documents in colored font. This updated document was submitted for peer review, and comments from 21 physicians and researchers were considered by the Panel in making revisions. The final document has been approved by the AUA PGC and the BOD. Funding of the Panel was provided by the AUA; members received no remuneration for their work. Each Panel member provided a conflict of interest disclosure to the AUA.

Evaluation goals

A couple attempting to conceive should have an evaluation for infertility if pregnancy fails to occur within one year of regular unprotected intercourse. An evaluation should be done before one year if 1) male infertility risk factors such as a history of bilateral cryptorchidism are known to be present; 2) female infertility risk factors, including advanced female age (over 35 years), are suspected; or 3) the couple questions the male partner's fertility potential. In addition, men who question their fertility status despite the absence of a current partner should have an evaluation of their fertility potential. The initial screening evaluation of the male partner of an infertile couple should include, at a minimum, a reproductive history and two semen analyses. If possible, the two semen analyses should be separated by a time period of at least one month. The reproductive history should include 1) coital frequency and timing; 2) duration of infertility and prior fertility; 3) childhood illnesses and developmental history; 4) systemic medical illnesses (e.g., diabetes mellitus and upper respiratory diseases) and prior surgeries; 5) sexual history including sexually transmitted infections; and 6) gonadal toxin exposure including heat. The semen analyses should be conducted as described in the section, 'Components of a full evaluation of male infertility.' While a man may have a history of previous fertility, this does not exclude the possibility that he has acquired a new, or secondary, male infertility factor. Men with secondary infertility should be evaluated in the same way as men who have never initiated a pregnancy (primary infertility).

Recommendations: An initial screening evaluation of the male partner of an infertile couple should be done if pregnancy has not occurred within one year of unprotected intercourse. An earlier evaluation may be warranted if a known male or female infertility risk factor exists or if a man questions his fertility potential. The

initial evaluation for male factor infertility should include a reproductive history and two properly performed semen analyses. A full evaluation by a urologist or other specialist in male reproduction should be done if the initial screening evaluation demonstrates an abnormal male reproductive history or an abnormal semen analysis. Further evaluation of the male partner should also be considered in couples with unexplained infertility and in couples in whom there is a treated female factor and persistent infertility.

When to do a full evaluation for infertility

The full evaluation for male infertility should include a complete medical and reproductive history, a physical examination by a urologist or other specialist in male reproduction and at least two semen analyses. Based on the results of the full evaluation, the physician may recommend other procedures and tests to elucidate the etiology of a patient's infertility. These tests may include additional semen analyses, endocrine evaluation, post-ejaculatory urinalysis, ultrasonography, specialized tests on semen and sperm, and genetic screening.

Components of a full evaluation for male infertility

Required evaluation components for every patient

Medical history

The patient's medical history is used to identify risk factors and behavior patterns that could have a significant impact on male infertility. The history should include all factors listed above for a reproductive history plus 1) a complete medical and surgical history; 2) a review of medications (prescription and non- prescription) and allergies; 3) a review of lifestyle exposures and a review of systems; 4) family reproductive history; and 5) a survey of past infections such as sexually transmitted diseases and respiratory infections.

Physical examination

A general physical examination is an integral part of the evaluation of male infertility. In addition to the general physical examination, particular focus should be given to the genitalia including 1) examination of the penis; including the location of the urethral meatus; 2) palpation of the testes and measurement of their size; 3) presence and consistency of both the vasa and epididymides; 4) presence of a varicocele; 5) secondary sex characteristics including body habitus, hair distribution and breast development; and 6) digital rectal exam. The diagnosis of congenital bilateral absence of the vasa deferentia (CBAVD) is established by physical examination. Scrotal exploration is not needed to make this diagnosis.

Semen analysis

Semen analysis is the cornerstone of the laboratory evaluation of the infertile male and helps to define the severity of the male factor. Methods of semen analysis are discussed in many textbooks, and detailed laboratory protocols have been published by the World Health Organization (WHO).[3] Physicians should provide patients with standard instructions for semen collection. These instructions should include a defined period of abstinence of two to three days. Semen can be collected by masturbation or by intercourse using special semen collection condoms that do not contain substances detrimental to sperm. The specimen may be collected at home or at the laboratory. The specimen should be kept at room or body temperature during transport and examined within one hour of collection. To ensure accurate results, the laboratory should have a quality control program for semen analysis, which conforms to the standards outlined in the Clinical Laboratory Improvement Amendments (CLIA). Information on these standards, which include proficiency testing, can be found at the CLIA web site.[4]

The semen analysis provides information on semen volume as well as sperm concentration, motility and morphology. Azoospermia should not be diagnosed until the specimen is centrifuged at maximum speed (preferably 3000 x g) for 15 minutes, and the pellet is examined. Although the methods for routine measurement of sperm concentration and motility have changed little during the past two decades, sperm morphology assessment has evolved considerably. The 1999 WHO criteria for scoring sperm morphology[3] are similar to the Kruger (Tygerberg) strict criteria .[5,6] When these criteria are applied to the evaluation of sperm morphology relatively few sperm are classified as having normal morphology, even in semen from fertile men. Sperm morphology assessment by strict criteria will be discussed later in depth and has been used to identify couples who have a poor chance of fertilization with standard in vitro fertilization (IVF)[5] or a better chance of fertilization with ICSI.[7] The WHO criteria of 1987 and 1992[8,9], which classify more sperm in the normal category, are also widely used in the routine semen evaluation. True reference ranges have not been established for semen parameters. The reference values in Table 1 are based on the clinical literature. Values that fall outside these ranges suggest a male infertility factor and indicate the need for additional clinical and/or laboratory evaluation of the patient. It must be emphasized that the reference values for semen parameters are not the same as the minimum values needed for conception, and that men with semen variables outside the reference ranges may be fertile. Conversely, patients with values within the reference range may still be infertile.

Recommendations: The minimum full evaluation for male infertility for every patient should include a complete medical history, physical examination by a urologist or other specialist in male reproduction and at least two semen analyses.

Additional procedures and tests, used to elucidate problems discovered by the full evaluation, may be suggested later as well.

Table 1: Semen Analysis: Reference Values

On at least two occasions:
Ejaculate volume 1.5-5.0 ml
pH >7.2
Sperm concentration >20 million/ml
Total sperm number >40 million/ejaculate
Percent motility >50%
Forward progression >2 (scale 0-4)
Normal morphology >50% normal* >30% normal** >14% normal***
And:
Sperm agglutination < 2 (Scale 0-3)
Viscosity <3 (Scale 0-4)

*World Health Organization, 1987[8].

**World Health Organization, 1992[9]

***Kruger (Tygerberg) Strict Criteria, World Health Organization, 1999.[1,5]

Other procedures and tests for assessing male fertility

Endocrine evaluation

Hormonal abnormalities of the hypothalamic-pituitary testicular axis are well-recognized, though not common causes of male infertility. An endocrine evaluation should be performed if there is: 1) an abnormal semen analysis, especially if the sperm concentration is less than 10 million/ml; 2) impaired sexual function; or 3) other clinical findings suggestive of a specific endocrinopathy. Some experts believe that all infertile males should have an endocrine evaluation, but there is no consensus of opinion on this controversy. The minimum initial hormonal evaluation should consist of measurements of serum follicle-stimulating-hormone (FSH) and serum testosterone levels. If the testosterone level is low, a repeat measurement of total and free testosterone (or bioavailable testosterone), as well as determination of serum luteinizing hormone (LH) and prolactin levels should be obtained. Although serum gonadotropin levels are variable because they are secreted in a pulsatile manner, a single measurement is usually sufficient to determine a patient's clinical endocrine status. The relationship of testosterone, LH, FSH and prolactin helps to identify the clinical condition (see Table 2). A normal serum FSH level does not guarantee the presence of intact spermatogenesis, however, an elevated FSH level even in the upper range of "normal" is indicative of an abnormality in spermatogenesis.

> **Recommendation: An initial endocrine evaluation should include at least a serum testosterone and FSH. It should be performed if there is: (1) an abnormally low sperm concentration, especially if less than 10 million/ml; (2) impaired sexual function; or (3) other clinical findings suggestive of a specific endocrinopathy.**

Table 2: The Relationship of Testosterone, LH, FSH and Prolactin with Clinical Condition				
Clinical Condition	**FSH**	**LH**	**Testosterone**	**Prolactin**
Normal spermatogenesis	Normal	Normal	Normal	Normal
Hypogonadotropic hypogonadism	Low	Low	Low	Normal
Abnormal spermatogenesis*	High/Normal	Normal	Normal	Normal
Complete testicular failure/ Hypergonadotropic hypogonadism	High	High	Normal/Low	Normal
Prolactin-secreting pituitary tumor	Normal/Low	Normal/Low	Low	High

* 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.

Post-ejaculatory urinalysis

Low-volume or absent ejaculate suggests retrograde ejaculation, lack of emission, ejaculatory duct obstruction, hypogonadism or CBAVD. In order to diagnose possible retrograde ejaculation, the physician should perform a post-ejaculatory urinalysis for any man whose ejaculate volume is less than 1.0 ml, and who has not been diagnosed with hypogonadism or CBAVD. It is also important to assure that either incomplete collection or very short abstinence periods (less than 1 day) are not the causes of the low-volume ejaculate. The post-ejaculatory urinalysis is performed by centrifuging the specimen for 10 minutes at a minimum of 300 x g, and microscopically inspecting the pellet at 400x magnification. The presence of *any* sperm in a post-ejaculatory urinalysis of a patient with azoospermia or aspermia is suggestive of the diagnosis of retrograde ejaculation. *Significant numbers* of sperm must be found in the urine of patients with low ejaculate volume oligospermia in order to suggest the diagnosis of retrograde ejaculation. Expert consensus on the definition of significant numbers of sperm in the urine does not exist.

Recommendation: A post-ejaculatory urinalysis should be performed in patients with ejaculate volumes of less than 1 ml, except in patients with bilateral vasal agenesis or clinical signs of hypogonadism.

Ultrasonography

Transrectal ultrasonography

Normal seminal vesicles are less than 2.0cm in anteroposterior diameter.[10] The finding of dilated seminal vesicles, dilated ejaculatory ducts and/or midline prostatic cystic structures on transrectal ultrasonography (TRUS) is suggestive of, but not diagnostic of, complete or partial ejaculatory duct obstruction.[11] Patients with complete ejaculatory duct obstruction produce low-volume, fructose negative, acidic, azoospermic ejaculates. Patients with CBAVD may also have these findings because they often have absent or atrophic seminal vesicles. Patients with partial ejaculatory duct obstruction often, but not always, present with semen having low volume, oligoasthenospermia and poor forward progression. Some experts routinely recommend TRUS in oligospermic patients with low volume ejaculates, palpable vasa and normal testicular size.

Recommendation: Transrectal ultrasonography is indicated in azoospermic patients with palpable vasa and low ejaculate volumes to determine if ejaculatory duct obstruction exists. Some experts recommend transrectal ultrasonography for oligospermic patients with low volume ejaculates, palpable vasa and normal testicular size to determine if partial ejaculatory duct obstruction is present.

Scrotal ultrasonography

Most scrotal pathology is palpable on physical examination. This includes varicoceles, spermatoceles, absence of the vasa, epididymal induration and testicular masses. Scrotal ultrasonography may identify non-palpable varicoceles, but these have not been shown to be clinically significant. Scrotal ultrasonography may be useful to clarify ambiguous findings on

examination, such as may occur in patients with testes that are in the upper scrotum, small scrotal sacs or other anatomy that makes physical examination of the scrotum difficult.

Recommendation: Scrotal ultrasonography is indicated in those patients in whom physical examination of the scrotum is difficult or inadequate or in whom a testicular mass is suspected.

Specialized clinical tests on semen and sperm

In some cases, semen analyses have failed to accurately predict a man's fertility. Therefore, there has been a search for other tests to improve the evaluation of the infertile male. Generally, these specialized clinical tests should be reserved only for those cases in which identification of the cause of male infertility will direct treatment.

Strict sperm morphology

The clinical implications of poor morphology scores remain highly controversial. Initial studies evaluating the utility of strict sperm morphology in predicting fertilization rates during IVF used a score of greater than 14% for normal. However, subsequent studies reported fertilization rates were lowest for patients with morphology scores of less than 4%. Pregnancy rates have also been reported to be suboptimal with lower scores[12] but some recent studies have reported no relationship of morphology to IVF results.[13] The relationship between morphology scores and pregnancy rates with intrauterine insemination (IUI)[14-16] and intercourse[17-18] have been examined, however, there has been no consensus on thresholds and management implications of poor morphology scores. Certain rare morphological abnormalities, such as sperm without acrosomes (globozoospermia), are highly predictive of failure to fertilize ova, yet in most cases fertilization and pregnancy are possible even with very low morphology scores. Although most clinicians utilize strict morphology in everyday practice, most studies have not addressed the

significance of isolated low morphology in patients with otherwise normal semen parameters. The current evidence suggests that, in general, sperm morphology scores should not be used in isolation to make patient management decisions.

Recommendation: Sperm morphology by rigid (strict) criteria has not been shown to be consistently predictive of fecundity and should not be used in isolation to make prognostic or therapeutic decisions.

DNA Integrity

DNA integrity testing refers to a variety of assays utilized to evaluate the degree of sperm DNA fragmentation. Assessment of sperm DNA integrity has been evaluated for correlation with inability to conceive by intercourse, IUI, IVF, and IVF using ICSI. In general, the assays demonstrate low sensitivity and high specificity. Studies of pregnancy by intercourse demonstrate a statistically significant lower pregnancy rate in those patients with impaired sperm DNA integrity, yet many couples with impaired sperm DNA integrity conceive by intercourse.[19-21] Currently the tests have inadequate sensitivity and specificity to be of value as screening tests for pregnancy by intercourse. One large study has suggested that abnormal DNA integrity in the sample used for IUI was highly predictive of pregnancy.[22] Without further studies, there is inadequate evidence to suggest the assay has prognostic value when performed prior to initiation of IUI. Most studies have examined the predictive value of sperm DNA integrity testing in routine IVF and IVF using ICSI. Meta-analysis of published studies has found a small statistically significant predictive effect of DNA integrity results upon pregnancy rates for IVF with or without ICSI.[23-24] However, the magnitude of the effect of an abnormal DNA integrity test on pregnancy rates is too small to warrant routine use of the test at this time. Limited data suggest DNA integrity testing may be of value in identifying those at risk for recurrent

pregnancy loss [25]. However there is insufficient evidence to warrant routine testing in these couples until further evidence accumulates. A variety of treatments have been suggested for patients with poor sperm DNA integrity, however there is no evidence demonstrating that treatment results in improved sperm DNA integrity and improved pregnancy/delivery rates.

> **Recommendation: Currently there is insufficient evidence in the literature to support the routine use of DNA integrity testing in the evaluation and management of the male partner of an infertile couple. Presently, there are no proven therapies to correct an abnormal DNA integrity test result**.

Reactive oxygen species (ROS)

Reactive oxygen species are 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. ROS also have a normal physiological role in the regulation of capacitation and the acrosome reaction. Elevated ROS have been implicated as a cause of male infertility. Controversy exists regarding the best method of testing for ROS; role of excess ROS in both natural conception and assisted reproductive technology; and whether therapies are effective at reducing seminal ROS and improving fecundity. Direct ROS testing is limited by the short duration of activity of the molecules. Seminal ROS is currently assessed by indirect testing methods that measure the products of ROS. Studies correlating seminal ROS levels to pregnancy outcomes are extremely limited or contradictory.[26-34] Most studies are limited by lack of controls and the lack of standardized testing methods for ROS makes comparison between studies difficult. The literature regarding ROS therapies is flawed by the paucity of randomized controlled trials and the lack of standardized type and duration of treatment. Review of the current literature reveals an inconsistent effect of therapies aimed at reducing seminal ROS upon

clinical parameters/outcomes. As a result routine clinical testing and treatment of ROS are not indicated at this time.

Recommendation: Reactive oxygen species testing has not been shown to be predictive of pregnancy independent of routine semen parameters nor are there any proven therapies to correct an abnormal test result. There is insufficient data to support the routine use of reactive oxygen species testing in the management of the male partner of an infertile couple.

Quantitation of leukocytes in semen

An elevated number of white blood cells in the semen has been associated with deficiencies in sperm function and motility. Under wet mount microscopy, both leukocytes and immature germ cells appear similar and are properly termed “round cells.” Many laboratories improperly report all round cells as “white blood cells.” The clinician must make sure that the two types of cells are differentiated. A variety of assays are available to differentiate leukocytes from immature germ cells. These include traditional cytologic staining and immunohistochemical techniques.[35] Those patients with true pyospermia (greater than 1 million leukocytes per ml) should be evaluated for a genital tract infection or inflammation.

Tests for antisperm antibodies

Pregnancy rates may be reduced by antisperm antibodies (ASA) in the semen.[36] Risk factors for ASA include ductal obstruction, prior genital infection, testicular trauma and prior vasovasostomy or vasoepididymostomy. ASA testing should be considered when there is isolated asthenospermia with normal sperm concentration, sperm agglutination or an abnormal postcoital test. Some physicians recommend ASA testing for couples with unexplained infertility. ASA found on the surface of sperm by direct testing are more significant than ASA

found in the serum or seminal plasma by indirect testing. ASA testing is not needed if sperm are to be used for ICSI.

Sperm viability tests

Sperm viability can be assessed by mixing fresh semen with a supravital dye such as eosin or trypan blue, or by the use of the hypoosmotic swelling (HOS) test .[3] These assays determine whether non-motile sperm are viable by identifying which sperm have intact cell membranes. Nonmotile but viable sperm, as determined by the HOS test, may be used successfully for ICSI.

Tests of sperm-cervical mucus interaction

The post-coital test is the microscopic examination of the cervical mucus, performed shortly before expected ovulation and within hours after intercourse, to identify the presence of motile sperm in the mucus. It is the traditional method for identifying cervical factors that contribute to infertility. Examination of the cervical mucus may reveal gross evidence of cervicitis that deserves treatment. However, abnormal cervical mucus or abnormal sperm/cervical mucus interaction is rarely the sole or principal cause of infertility. Furthermore, controversies exist regarding technique, timing and interpretation of this test. Results of the post-coital test are subjective and exhibit considerable intra- and inter-observer variation. Although its utility and predictive value have been seriously questioned[37], some practitioners still consider it a useful diagnostic test[38] because it may help to identify ineffective coital technique or a cervical factor not otherwise suspected on the basis of history and physical examination. Contemporary treatments for otherwise unexplained infertility, such as superovulation and intrauterine insemination or in vitro fertilization, effectively negate any unrecognized cervical factors. Routine postcoital testing is unnecessary. The test may be reserved for patients in whom results will influence treatment strategy.

Zona free hamster oocyte test

Removal of the zona pellucida from hamster oocytes allows human sperm to fuse with hamster ova. This test is often termed a sperm penetration assay (SPA). This test should also be reserved for patients in whom results will influence treatment strategy. For penetration to occur, sperm must undergo capacitation, the acrosome reaction, fusion with the oolemma and incorporation into the ooplasm. Many versions of the SPA have been used clinically[3, 39], and the value of the test results depends, in part, on the experience of the laboratory performing the assay.

Computer-aided sperm analysis

Computer-aided sperm analysis (CASA) requires sophisticated instruments for quantitative assessment of sperm from a microscopic image or from videotape. In principle, CASA can be used to objectively measure sperm numbers, motility and morphology. CASA instruments are most useful clinically for assessing sperm motility and motion parameters, such as velocity or speed and head movement, which some believe may be important factors in determining sperm fertility potential.

Recommendation: Specialized tests on semen are not required for diagnosis of male infertility. They may be useful in a small number of patients for identifying a male factor contributing to unexplained infertility, or for selecting therapy, such as assisted reproductive technology.

Less commonly used specialized tests

In addition to the zona-free hamster oocyte tests, numerous tests of sperm function have been employed in research studies. The acrosome reaction of human sperm can be detected using specialized staining techniques. Rates of spontaneous acrosome reactions and acrosome reactions induced by agents such as calcium ionophore and progesterone have been measured. Samples from infertile men tend to demonstrate lower induced acrosome reaction levels than fertile

men.[40] In addition, sperm function can be evaluated using human zona pellucida binding tests. In some cases, these tests have detected a probable cause for low fertilization rates or failed IVF.[41]

Recommendation: Less commonly used specialized tests on semen are important investigative tools, but are not necessary for the routine evaluation of men with infertility.

Genetic screening

Genetic abnormalities may cause infertility by affecting sperm production or sperm transport. The three most common genetic factors known to be related to male infertility are: 1) cystic fibrosis gene mutations associated with congenital absence of the vas deferens; 2) chromosomal abnormalities resulting in impaired testicular function; and 3) Y-chromosome microdeletions associated with isolated spermatogenic impairment. Azoospermia and severe oligospermia may be associated with genetic abnormalities. Men with nonobstructive azoospermia and severe oligospermia should be informed that they might have chromosomal abnormalities or Y-chromosome microdeletions. In addition, men with azoospermia due to CBAVD should be informed that they probably have an abnormality of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Genetic counseling should be provided whenever a genetic abnormality is detected.

Cystic fibrosis gene mutations

The most common cause of CBAVD is a mutation of the CFTR gene. Almost all males with clinical cystic fibrosis have CBAVD. Approximately 70% of men with CBAVD and no clinical evidence of cystic fibrosis have an identifiable abnormality of CFTR gene.[42-43] Since normal vasa are easily palpable within the scrotum, the diagnosis of vasal agenesis, either bilateral or unilateral, is established by physical examination. Imaging studies and surgical exploration are

not necessary to confirm the diagnosis, but may be useful for diagnosing abnormalities associated with vasal agenesis. Most patients with vasal agenesis also have seminal vesicle hypoplasia or agenesis. Since the majority of semen is derived from the seminal vesicles, almost all patients with CBAVD have low semen volume. In the azoospermic patient who has unilateral vasal agenesis, radiologic imaging with transrectal ultrasonography (TRUS) may be useful to evaluate the ampullary portion of the contralateral vas deferens and the seminal vesicles, because unilateral vasal agenesis can be associated with contralateral segmental atresia of the vas deferens or seminal vesicle, resulting in obstructive azoospermia.[44]

It is recommended that both partners undergo genetic counseling and testing of the CFTR gene to rule out abnormalities. Failure to identify a CFTR abnormality in a man with CBAVD, however, does not absolutely rule out the presence of a mutation, since many are undetectable by routine testing methods. Since it can be assumed that many men with CBAVD harbor a genetic abnormality in the CFTR gene, whether or not their testing is positive, it is important to test the spouse for CFTR gene abnormalities prior to performing a treatment that utilizes his sperm because of the risk (approximately 4% in North American Caucasians) that she may be a carrier. Ideally, genetic counseling should be offered both before and after genetic testing of both partners. The main arguments for genetic testing of the patient with CBAVD is that this information is important for counseling the patient regarding future health effects of CFTR mutations[45-46]as well as counseling siblings about their risk of being carriers of CFTR mutations. There is a strong association between unilateral vasal agenesis and ipsilateral renal anomalies due to their common embryological origin. In contrast, the association of renal anomalies and congenital bilateral absence of the vasa deferentia (CBAVD) is much weaker with a prevalence

of only 11%. However, for those patients who have CBAVD and CFTR mutations the prevalence of renal anomalies is extremely rare.[47] Therefore, imaging of the kidneys with either ultrasound or CT scan is more likely to detect abnormalities in men with unilateral vasal agenesis or men with CBAVD who do not have mutations in CFTR.

Recommendations: Men with congenital bilateral absence of the vasa deferentia should be offered genetic counseling and testing for cystic fibrosis transmembrane conductance regulator mutations. The female partner should also be offered cystic fibrosis transmembrane conductance regulator mutations testing before proceeding with treatments that utilize the sperm of a man with congenital bilateral absence of the vasa deferentia. Imaging for renal abnormalities should be offered to men with unilateral vasal agenesis or congenital bilateral absence of the vasa deferentia and no evidence of cystic fibrosis transmembrane conductance regulator abnormalities.

Cystic fibrosis transmembrane conductance regulator testing

Routine screening for mutations of CFTR is currently performed by testing for a panel of specific mutations that are known to be prevalent rather than sequencing the entire gene. The CFTR gene is extremely large and the number of mutations potentially infinite. Clinical laboratories typically test for the 30–50 most common mutations found in patients with clinical cystic fibrosis. However, the mutations associated with CBAVD may be different. There are more extended panels available that test up to 100 mutations. Because over 1,300 different mutations have been identified in this gene, this type of limited analysis is only informative if a mutation is found. A negative test result only indicates that the CBAVD patient does not have the most common mutations causing cystic fibrosis. Direct sequence analysis of the entire gene is commercially available but very costly. In addition to point mutations, variations of intron 8

of the CFTR gene where repeat sequences act as a rheostat controlling the expression of the CFTR protein can result in an abnormal phenotype. For instance, a polythymidine sequence located at the end of intron 8 that is only 5 bases (5T allele) long, rather than 7 or 9, exacerbates skipping of exon 9, thereby reducing the production of functional protein. In addition, there is an inverse relationship between the lengths of an adjacent thymidine-guanine (TG) repeat sequence and expression of the CFTR protein when the 5T variant is present. Those individuals with the 5T variant adjacent to either 12 or 13 TG repeats are significantly more likely to exhibit CBAVD than individuals with only 11 TG repeats.[48-50] Variants in the number of TG repeats only decreases CFTR production when the 5T allele is present, therefore testing for the TG repeat in a patient without the 5T variant has no clinical implication.

Recommendations: Testing for cystic fibrosis transmembrane conductance regulator abnormalities should include at minimum a panel of common point mutations and the 5T allele.

There currently is no consensus on the minimum number of mutations that should be tested.

Gene sequencing may be considered in couples where the wife is a carrier and the husband with congenital bilateral absence of the vasa deferentia tests negative on a routine panel of cystic fibrosis transmembrane conductance regulator mutations.

Genetic testing

A male presenting with infertility is more likely than the general population to harbor a gene mutation or chromosomal abnormality. Indeed, up to 15% of men with azoospermia have an abnormality in their karyotype[51-54], Y chromosome microdeletion[55-56], or mutation in the cystic fibrosis transmembrane conductance regulating (CFTR) gene.

Karyotype

A karyotype analyzes all chromosomes for the gain or loss of entire chromosomes as well as structural defects, including chromosome rearrangements (translocations), duplications, deletions, and inversions. Chromosome abnormalities account for about 6% of all male infertility, and the prevalence increases with increased spermatogenic impairment (severe oligospermia and nonobstructive azoospermia). Paternal transmission of chromosome defects can result in pregnancy loss, birth defects, male infertility, and other genomic syndromes.

Recommendation: Karyotyping and genetic counseling should be offered to all patients with nonobstructive azoospermia and severe oligospermia (<5 million sperm/ml).

Y-chromosome microdeletions

Approximately 13 % of men with nonobstructive azoospermia or severe oligospermia have an underlying Y-chromosome microdeletion.[57] Y chromosome microdeletions responsible for infertility — regions AZF a, b, or c — are detected using sequence tagged sites (STS) and polymerase chain reaction (PCR) analysis. There is no consensus on the number of STS required for optimal detection of AZF deletions. Detection has both prognostic and ethical significance. Successful testicular sperm extraction has not been reported in infertile men with either an AZFa or AZFb deletion but the total number of reports is limited.[58] In contrast, up to 80% of men with AZFc deletions have retrievable sperm for ICSI. Furthermore, the couple must be counseled on the inheritance of this compromised fertility potential in all male offspring.[59-60]

Recommendation: There are insufficient data to recommend a minimal number of sequence tagged sites to test for in patients undergoing Y chromosome microdeletion analysis. Although the prognosis for sperm retrieval is poor in

patients having large deletions involving AZF region a or b, the results of Y chromosome deletion analysis cannot absolutely predict the absence of sperm.

Conflict of Interest Disclosure

All panel members completed Conflict of Interest disclosure. Those marked with (C) indicate that compensation was received; relationships designated by (U) indicate no compensation was received; (A) indicates affiliation.

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Acknowledgements and Disclaimers

The Optimal Evaluation of the Infertile Male: Best Practice Statement

The supporting systematic literature review and the drafting of this document were conducted by the Infertility Best Practice Statement Panel (the Panel) created in 2007 by the AUA. The PGC of the AUA selected the Panel chair who in turn appointed the additional Panel members with specific expertise in evaluation of the infertile male. The mission of the Panel was to develop either analysis- or consensus-based recommendations, depending on the type of evidence available and Panel processes, to support optimal clinical practices concerning the infertile male. This document was submitted to 58 urologists and other health care professionals for peer review. After revision of the document based upon the peer review comments, the best practice statement was submitted to and approved by the PGC and the BOD of the AUA. Funding of the Panel and of the PGC was provided by the AUA. Panel members received no remuneration for their work. Each member of the PGC and of the Panel furnished a current conflict of interest disclosure to the AUA. The final report is intended to provide medical practitioners with a current understanding of the principles and strategies for the optimal evaluation of the infertile male. The report is based on review of available professional literature as well as clinical experience and expert opinion. This document provides guidance only and does not establish a fixed set of rules or define the legal standard of care. As medical knowledge expands and technology advances, this best practice statement will change. Today they represent not absolute mandates but provisional proposals or recommendations for treatment under the specific conditions described. For all these reasons, this best practice statement does not preempt physician judgment in individual cases. Also, treating physicians must take into account

variations in resources, and in patient tolerances, needs and preferences. Conformance with the best practice statement reflected in this document cannot guarantee a successful outcome.

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References

1. Thonneau P, Marchand S, Tallec A et al: Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988-1989). Hum Reprod 1991; **6**:811.
2. Honig SC, Lipshultz LI and Jarow J: Significant medical pathology uncovered by a comprehensive male infertility evaluation. Fertil Steril 1994; **62**:1028.
3. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction; 4th edition, Cambridge University Press, 1999
4. CLIA website: wwwn.cdc.gov/clia/default.aspx
5. Kruger TF, Acosta AA, Simmons KF et al: Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil Steril 1988; **49**:112.
6. Menkveld R, Stander FS, Kotze TJ et al: The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. Hum Reprod 1990; **5**:586.
7. Pisarska MD, Casson, PR, Cisneros PL et al: Fertilization after standard in vitro fertilization versus intracytoplasmic sperm injection in subfertile males using sibling oocytes. Fertil Steril 1999; **71**:627.
8. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction; 2nd edition, Cambridge University Press, 1987.
9. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction; 3rd edition, Cambridge University Press, 1992.
10. Carter SS, Shinohara K and Lipshultz LI: Transrectal ultrasonography in disorders of the seminal vesicles and ejaculatory ducts. Urol Clin North Am 1989; **16**:773.
11. Jarow JP: Transrectal ultrasonography of infertile men. Fertil Steril 1993; **60**:1035.

12. Coetzee K, Kruge TF and Lombard C J: Predictive value of normal sperm morphology: a structured literature review. Hum Reprod Update 1998; **4:** 73.

13. Keegan BR, Barton S, Sanchez X et al: Isolated teratozoospermia does not affect in vitro fertilization outcome and is not an indication for intracytoplasmic sperm injection. Fertil Steril 2007; **88:** 1583.

14. Van Waart J, Kruger TF, Lombard CJ et al: Predictive value of normal sperm morphology in intrauterine insemination (IUI): a structured literature review. Hum Reprod Update 2001; **7:** 495.

15. Spiessens C, Vanderschueren D, Meuleman C et al: Isolated teratozoospermia and intrauterine insemination. Fertil Steril 2003; **80:** 1185.

16. Shibahara H, Obara H, Ayustawati et al: Prediction of pregnancy by intrauterine insemination using CASA estimates and strict criteria in patients with male factor infertility. Int J Androl 2004, **27:** 63.

17. Gunalp S, Onculoglu C, Gurgan T et al: A study of semen parameters with emphasis on sperm morphology in a fertile population: an attempt to develop clinical thresholds. Hum Reprod 2001; **16:** 110.

18. Guzick DS, Overstreet JW, Factor-Litvak P et al.: Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med 2001; **345:** 1388.

19. Evenson DP, Jost LK, Marshall D et al: Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod 1999; **14:** 1039.

20. Spano M, Bonde JP, Hjollund HI et al: Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. Fertil Steril 2000, **73:** 43.

21. Evenson DP and Wixon R: Data analysis of two in vivo fertility studies using Sperm Chromatin Structure Assay-derived DNA fragmentation index vs. pregnancy outcome. Fertil Steril 2008; **90:** 1229.

22. Bungum M, Humaidan P, Axmon A et al: Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod 2007; **22:** 174.

23. Collins JA, Barnhart KT and Schlegel PN: Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? Fertil Steril 2008; **89:** 823.

24. Zini A and Sigman M. Are tests of sperm DNA damage clinically useful? Pros and cons. J Androl. 2009; **30**:219.

25. Zini A, Boman JM, Belzile E et al.: Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod. 2008; **23**:2663.

26. Aitken RJ, Irvine DS and Wu FC: Prospective analysis of sperm-oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility. Am J Obstet Gynecol 1991; **164:** 542.

27. Sukcharoen N, Keith J, Irvine DS et al: Prediction of the in-vitro fertilization (IVF) potential of human spermatozoa using sperm function tests: the effect of the delay between testing and IVF. Hum Reprod 1996, **11:** 1030.

28. Krausz C, Mills C, Rogers S et al: Stimulation of oxidant generation by human sperm suspensions using phorbol esters and formyl peptides: relationships with motility and fertilization in vitro. Fertil Steril 1994; **62:** 599.

29. Marchetti C, Obert G, Deffosez A et al: Study of mitochondrial membrane potential, reactive oxygen species, DNA fragmentation and cell viability by flow cytometry in human sperm. Hum Reprod 2002; **17:** 1257.

30. Yeung CH, De GC, De GM et al: Production of reactive oxygen species by and hydrogen peroxide scavenging activity of spermatozoa in an IVF program. J Assist Reprod Genet 1996; **13:** 495.

31. Moilanen JM, Carpen O and Hovatta O: Flow cytometric light scattering analysis, acrosome reaction, reactive oxygen species production and leukocyte contamination of semen preparation in prediction of fertilization rate in vitro. Hum Reprod 1998; **1**3**:** 2568.

32. Zorn B, Vidmar G and Meden-Vrtovec H: Seminal reactive oxygen species as predictors of fertilization, embryo quality and pregnancy rates after conventional in vitro fertilization and intracytoplasmic sperm injection. Int J Androl 2003; **26:** 279.

33. Saleh RA, Agarwal A, Nada EA et al.: Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. Fertil Steril 2003; **79:** 1597.

34. Hammadeh ME, Radwan M, Al-Hasani S et al.: Comparison of reactive oxygen species concentration in seminal plasma and semen parameters in partners of pregnant and non-pregnant patients after IVF/ICSI. Reprod Biomed Online 2006; **13:** 696.

35. Wolff H and Anderson DJ. Immunohistologic characterization and quantitation of leukocyte subpopulations in human semen. Fertil Steril 1988; **49**:497.

36. Ayvaliotis B, Bronson R, Rosenfeld D et al. Conception rates in couples where autoimmunity to sperm is detected. Fertil Steril 1985;**43**:739.

37. Oei SG, Helmerhorst FM, Bloemenkamp KM et al.: Effectiveness of the postcoital test: randomised controlled trial. BMJ 1998; **317**:502.

38. Glatstein IZ, Harlow BL and Hornstein MD.: Practice patterns among reproductive endocrinologists: further aspects of the infertility evaluation. Fertil Steril 1998;**70**:263.

39. Smith RG, Johnson A, Lamb D et al.: Functional tests of spermatozoa. Sperm penetration assay. Urol Clin North Am 1987;**14**:451.

40. Liu de Y, Liu ML, Garrett C et al: Comparison of the frequency of defective sperm-zona pellucida (ZP) binding and the ZP-induced acrosome reaction between subfertile men with normal and abnormal semen. Hum Reprod. 2007; **22**: 1878. Epub 2007 Apr

41. Franken DR, Oehninger S, Burkman LJ et al: The hemizona assay (HZA): a predictor of human sperm fertilizing potential in in vitro fertilization (IVF) treatment. J In Vitro Fert Embryo Transf 1989;**6**:44.

42. Anguiano A, Oates RD, Amos JA et al: Congenital bilateral absence of the vas deferens. A primarily genital form of cystic fibrosis. JAMA 1992; **267**:1794.

43. Chillon M, Casals T, Mercier B et al: Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. New Engl J Med 1995; **332**: 1475.

44. Hall S and Oates RD: Unilateral absence of the scrotal vas deferens associated with contralateral mesonephric duct anomalies resulting in infertility: laboratory, physical and radiographic findings, and therapeutic alternatives. J Urol 1993; **150**:1161.

45. Castellani C., Bonizzato A, Pradal U et al: Evidence of mild respiratory disease in men with congenital absence of the vas deferens. Respiratory Medicine 1999;**93**:869.

46. Gilljam M, Moltyaner Y, Downey GP et al: Airway inflammation and infection in congenital absence of the vas deferens. Am J Respir Crit Care Med 2004;**169**:174.

47. Schlegel PN, Shin D and Goldstein M: Urogenital anomalies in men with congenital absence of the vas deferens. J Urol 1996; **155**:1644.

48. Dörk T, Dworniczak B, Aulehla-Scholz C et al: Distinct spectrum of CFTR gene mutations in congenital absence of vas deferens. Hum Genet 1997; **100**:365.

49. Costes B, Girodon E, Ghanem N et al.: Frequent occurrence of the CFTR intron 8 (TG)n 5T allele in men with congenital bilateral absence of the vas deferens. Eur J Hum Genet 1995; **3**:285.

50. Viel M, Leroy C, Des Georges M et al: Novel length variant of the polypyrimidine tract within the splice acceptor site in intron 8 of the CFTR gene: Consequences for genetic testing using standard assays. Eur J Hum Genet 2005; **13**:136.

51. Bourrouillou G, Bujan L, Calvas P et al: Role and contribution of karyotyping in male infertility. Prog Urol 1992; **2**:189.

52. Rao MM and Rao DM: Cytogenetic studies in primary infertility. Fertil Steril 1977; **28**:209.

53. Gekas J, Thepot F, Turleau C et al.: Chromosomal factors of infertility in candidate couples for ICSI: An equal risk of constitutional aberrations in women and men. Hum Reprod 2001; **16**:82.

54. Van AE, Bonduelle M, Tournaye H et al.: Cytogenetics of infertile men. Hum Reprod 1996; **11**:1.

55. Seifer I, Amat S, Delgado-Viscogliosi P et al: Screening for microdeletions on the long arm of chromosome Y in 53 infertile men. Int J Androl 1999; **22**:148.

56. Nakahori Y, Kuroki Y, Komaki R et al: The Y chromosome region essential for spermatogenesis. Horm Res 1996; **46**:20.

57. Reijo R, Alagappan RK, Patrizio P et al: Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. Lancet 1996; **347**:1290.

58. Hopps CV, Mielnik A, Goldstein M et al: Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. Hum Reprod 2003; **18**:1660.

59. Silber SJ and Repping S: Transmission of male infertility to future generations: Lessons from the Y chromosome. Hum Reprod Update 2002; **8**:217.

60. Foresta C, Moro E and Ferlin A: Y chromosome microdeletions and alterations of spermatogenesis. Endocr Rev 2001; **22**:226.

This best practice statement is intended to provide medical practitioners with a consensus of principles and strategies for the care of couples with male infertility problems. The document is based on current professional literature, clinical experience and expert opinion. It does not establish a fixed set of rules or define the legal standard of care and it does not preempt physician judgment in individual cases. Physician judgment must take into account variations in resources and in patient needs and preferences. Conformance with this best practice statement cannot ensure a successful result.

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