Report on evaluation of the azoospermic male

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INTRODUCTION

Approximately 15 percent of couples are unable to conceive after one year of unprotected intercourse. A male factor is solely responsible in about 20 percent of infertile couples and contributory in another 30-40 percent (1). Azoospermia, defined as complete absence of sperm from the ejaculate, is present in about 1 percent of all men (2) and in 10 to 15 percent of infertile men (3). Azoospermia is different from aspermia, in that aspermia is the complete absence of seminal fluid emission upon ejaculation. Differentiation of azoospermia from severe oligospermia is accomplished by examination of the pellet of a centrifuged semen sample on at least two occasions.

This review offers recommendations for diagnosing and defining the etiology of azoospermia. Patients with severe oligospermia may be evaluated in a similar manner.

INITIAL DIAGNOSIS OF AZOOSPERMIA

The initial diagnosis of azoospermia is made when no spermatozoa can be detected on high-powered microscopic examination of centrifuged seminal fluid on at least two occasions. The WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction recommends that the seminal fluid be centrifuged for 15 minutes at a centrifugation speed of, preferably, 3000g or greater (4).

Recommendation: The diagnosis of azoospermia requires the absence of sperm from at least two separate centrifuged semen samples.

DIFFERENTIAL DIAGNOSIS OF THE AZOOSPERMIC PATIENT

The evaluation of a patient with azoospermia is performed to determine the etiology of the patient’s condition. This allows the physician to: 1) establish whether the cause of azoospermia is amenable to therapy; 2) identify appropriate treatment options; and 3) determine whether a significant medical disorder is the underlying cause of the azoospermia.

The numerous etiologies for azoospermia fall into three categories: pre-testicular, testicular and post-testicular. Pre-testicular causes of azoospermia are endocrine abnormalities that adversely affect spermatogenesis (secondary testicular failure) and are relatively rare. Testicular etiologies (primary testicular failure) involve disorders of spermatogenesis intrinsic to the testes. Post-testicular etiologies of azoospermia are due to either ejaculatory dysfunction or obstruction of sperm delivery to the urethral meatus, and are found in approximately 40% of patients (3). The pre-testicular and post-testicular abnormalities that cause azoospermia are frequently correctable. Testicular disorders are generally irreversible, with the possible exception of impaired spermatogenesis associated with varicoceles.

INITIAL EVALUATION OF THE AZOOSPERMIC PATIENT

To help differentiate between reversible and irreversible causes of azoospermia, the minimum initial evaluation of an azoospermic patient should include a complete medical history, physical examination and hormone level measurements. Relevant history includes: 1) prior fertility; 2) childhood illnesses such as...
Azoospermia may be caused by either primary or secondary testicular failure. The results of the initial endocrine tests are used to distinguish between these two possibilities. An elevated serum FSH level associated with bilaterally small testes and a low serum testosterone level is consistent with primary testicular failure. All patients with these findings should be offered genetic testing for chromosomal abnormalities and Y-chromosome microdeletions. A separate, detailed discussion of genetic testing for men with azoospermia appears later in this document. A low serum FSH level associated with bilaterally small testes and a low serum testosterone level is consistent with hypogonadotropic hypogonadism. These patients usually have low serum luteinizing hormone (LH) levels as well. Hypogonadotropic hypogonadism can be caused by hypothalamic disorders, e.g., Kallmann syndrome, or congenital or acquired pituitary disorders, e.g., functioning and non-functioning pituitary tumors. Therefore, these patients should undergo further evaluation, including serum prolactin level measurement and imaging of the pituitary.

**Recommendations:** All patients with azoospermia due to primary hypogonadism should be offered genetic testing. Patients with acquired hypogonadotropic hypogonadism should be evaluated for functioning and non-functioning pituitary tumors by measurement of serum prolactin and imaging of the pituitary gland.

**Ductal Obstruction**

When vasal agenesis and testicular atrophy are not present, semen volume and serum FSH are key factors in determining the etiology of the azoospermia. Azoospermic
patients with normal ejaculate volume may have either ob-
struction of the reproductive system or abnormalities of
spermatogenesis. Azoospermic patients with low semen vol-
ume and normal sized testes may have ejaculatory dysfunc-
tion or ejaculatory duct obstruction.

**Patients with Normal Ejaculate Volume**

The serum FSH of a patient with normal semen volume is a critical factor in determining whether a diagnostic testicu-
lar biopsy is needed to establish the presence or absence of
normal spermatogenesis (9). Marked elevation of serum FSH (greater than two times the upper limit of normal) is diag-
nostic of abnormal spermatogenesis. Therefore, a diagnostic
testicular biopsy is not necessary in these patients. However,
if sperm retrieval with ICSI is being considered, a testicular
biopsy may be performed for prognostic purposes, to deter-
mine whether spermatooza are likely to be retrievable by
future testicular sperm aspiration or extraction. The presence
or absence of sperm in a biopsy specimen, however, does not
absolutely predict whether sperm are present elsewhere
within that testicle. Therefore, controversy exists among
experts regarding the role of prognostic biopsy in a patient
with a markedly elevated serum FSH.

Conversely, patients who have a normal serum FSH
should undergo a diagnostic testicular biopsy, as a normal
serum FSH level does not assure the presence of normal
spermatogenesis. It is acceptable to perform either a unilat-
eral or bilateral testicular biopsy in these patients, as there is
currently no clear consensus on this issue. If a unilateral
biopsy is undertaken, it should be performed on the larger
testis.

Testicular biopsy can be performed by a standard open
incision technique or by percutaneous methods. A routine
open testicular biopsy, performed under local anesthesia, is
the most common method. This should be performed
through a small scrotal incision without delivering the testis
outside the skin or tunica vaginalis. This minimizes postop-
erative scarring and therefore facilitates subsequent scrotal
reconstructive surgery. The testicular biopsy specimen
should be placed in an appropriate fixative such as Bouin’s,
Zenker’s or glutaraldehyde. Formalin should not be used. At
the time of a diagnostic or prognostic biopsy, it is possible to
obtain a portion of testicular tissue for cryopreservation and
use in a future IVF/ICSI cycle, thus obviating the need for a
second surgery.

If the testicular biopsy is normal, obstruction at some
level in the reproductive system must be present and the
location of the obstruction may then be determined. Most
men with obstructive azoospermia and no history suggesting
iatrogenic vasal injury have bilateral epididymal obstruction.
Epididymal obstruction can be identified only by surgical
exploration. Vasography may be utilized to determine
whether there is an obstruction in the vas deferens or ejac-
ulatory ducts. Because of the risk of vasal scarring and
obstruction, vasography should not be performed at the time
of diagnostic testicular biopsy, unless reconstructive surgery
is undertaken at the same time.

**Recommendations:** In order to distinguish between ob-
structive and nonobstructive causes of azoospermia, diag-
nostic testicular biopsy is indicated for patients with normal
testicular size, at least one palpable vas deferens and a
normal serum FSH level. Vasography should not be per-
formed at the time of diagnostic testicular biopsy unless
reconstructive surgery is undertaken at the same time.

**Patients with Low Ejaculate Volume**

Low ejaculate volume (< 1.0 ml) that is not caused by
hypogonadism or CBAVD (see previous sections) can be
caused by ejaculatory dysfunction, but is most likely caused
by ejaculatory duct obstruction (EDO). Ejaculatory dysfunc-
tion rarely, if ever, causes low ejaculate volume with
azoospermia, although it is a well-known cause of aspermia
or low ejaculate volume with oligospermia. Additional sem-
inal parameters that can be helpful in determining the pres-
ence of EDO are seminal pH and fructose, since the seminal
vesicle secretions are alkaline and contain fructose. How-
ever, the results of semen pH and fructose testing may be
misleading when these tests are not properly performed and,
therefore, many experts tend to give less weight to these
parameters over other clinical findings.

Transrectal ultrasonography (TRUS) is indicated for the
diagnosis of EDO in men with low ejaculate volume. While
vasography is an alternative diagnostic test for EDO, TRUS
is minimally invasive and avoids the risk of vasal injury
associated with vasography (10). The finding of midline
cysts, dilated ejaculatory ducts and/or dilated seminal ves-
icles (greater than 1.5 cm in anteroposterior diameter) on
TRUS is suggestive, but not diagnostic, of ejaculatory duct
obstruction (11, 12). Conversely, normal seminal vesicle size
does not completely rule out the possibility of obstruction.
Therefore, seminal vesicle aspiration (SVA) and seminal
vesiculography may be performed under TRUS guidance to
make a more definitive diagnosis of EDO (13). The presence
of large numbers of sperm in the seminal vesicle of an
azoospermic patient is highly suggestive of EDO. Seminal
vesiculography performed concurrently with SVA can de-
termine the anatomic site of the obstruction. Vasography
with simultaneous examination of intravasal fluid for sperm,
and simultaneous testicular biopsy constitute the alternative
approach for diagnosing ejaculatory duct obstruction in the
patient with low-ejaculate-volume azoospermia.

**Recommendation:** Testicular biopsy may be performed
to confirm the presence of reproductive tract obstruction in
patients with low ejaculate volume azoospermia and palpable
vasa. Transrectal ultrasonography, with or without sem-
inal vesicle aspiration and seminal vesiculography, may be
used to identify obstruction in the distal male reproductive
tract. Alternatively, vasography may be used to identify the
sites of reproductive tract obstruction in patients with low ejaculate volume azoospermia and palpable vasa but should not be done unless reconstructive surgery is undertaken at the same surgical procedure.

Genetic Testing in Patients with Azoospermia

In addition to mutations in the CFTR gene that give rise to CBAVD, genetic factors may play a role in nonobstructive forms of azoospermia. The two most common categories of genetic factors associated with nonobstructive azoospermia are: 1) chromosomal abnormalities resulting in impaired testicular function; and 2) Y-chromosome microdeletions leading to isolated spermatogenic impairment.

Karyotypic Chromosomal Abnormalities

Chromosomal abnormalities that can be observed on karyotypes of peripheral leukocytes are present in approximately 7 percent of infertile men. The frequency of karyotypic abnormalities is inversely proportional to the sperm count; with a prevalence of 10-15 percent in azoospermic men, approximately 5 percent in oligospermic men and less than 1 percent in normospermic men (14). Sex chromosomal aneuploidy (Klinefelter syndrome) accounts for approximately two-thirds of chromosomal abnormalities observed in infertile men. Structural abnormalities of the autosomal chromosomes, such as inversions and translocations, are also observed at a higher frequency in infertile men than in the general population. When the male has gross karyotypic abnormalities, the couple is at increased risk for miscarriages and for having children with chromosomal and congenital defects. Karyotyping should be offered to men who have nonobstructive azoospermia or severe oligospermia prior to performing ICSI with their sperm.

Y-Chromosome Microdeletions

Microdeletions of the Y chromosome may be found in 10-15 percent of men with azoospermia or severe oligospermia (15). These microdeletions are too small to be detected by karyotyping but can be found by using polymerase chain reaction (PCR) techniques to analyze sequence-tagged sites that have been mapped along the entire length of the Y chromosome. Most deletions causing azoospermia or oligospermia occur in non-overlapping regions of the long arm of the Y chromosome (Yq11). These regions have been designated as AZFa (proximal), AZFb (central), and AZFc (distal). It appears that these regions of the Y chromosome contain multiple genes necessary for spermatogenesis. The DAZ (deleted in azoospermia) gene, for example, which encodes a transcription factor that is usually present in men with normal fertility, is located in the AZFc region.

The specific location of the deletion along the Y chromosome may significantly affect spermatogenesis. If the deleted region of the Y chromosome is in the AZFc region, sperm will be present in the ejaculate in many patients, albeit in severely reduced numbers. Other patients with AZFc region deletions will be azoospermic but still may have sperm production that is sufficient to allow sperm extraction by testis biopsy. The presence of a deletion involving the entire AZFb region, however, appears to predict a very poor prognosis for sperm retrieval despite extensive testicular biopsies (16). Poor sperm retrieval results may also exist for men with deletions involving the AZFa region (17).

Sons of individuals with a Y-chromosome microdeletion will inherit the microdeletion and may consequently be infertile (18). Although a microdeletion of the Y chromosome is not thought to be associated with other health problems, few data exist on the phenotypes of the sons of fathers with such genetic abnormalities. It is important to note that a negative Y-chromosome microdeletion assay does not necessarily rule out a genetic abnormality, because there may be other presently unknown gene sequences on the Y or other chromosomes that might also be necessary for spermatogenesis. Conversely, it has been shown that some Y-chromosome microdeletions may be found in fertile or sub-fertile males who have fathered children (15, 19). Y-chromosome analysis should be offered to men who have non-obstructive azoospermia or severe oligospermia prior to performing ICSI with their sperm.

Recommendations: Men with non-obstructive azoospermia and severe oligospermia should be informed of the potential genetic abnormalities associated with azoospermia or severe oligospermia.

Karyotyping, Y-chromosome analysis and genetic counseling should be offered to men with non-obstructive azoospermia prior to performing ICSI with their sperm. Genetic counseling may be offered whenever a genetic abnormality is suspected in either the male or the female partner and should be provided whenever a genetic abnormality is detected.

References

How This Document Was Created

This document was written by the Male Infertility Best Practice Policy Committee of the American Urological Association, Inc.® (AUA) and the Practice Committee of the American Society for Reproductive Medicine (ASRM). The two organizations agreed to collaborate to prepare documents of importance in the field of male infertility. The Male Infertility Best Practice Policy Committee was created in 1999 by the Board of Directors of the American Urological Association, Inc.® The Committee co-chairmen and members were selected by the Practice Parameters, Guidelines and Standards Committee (PPGSC) of the AUA. The membership of the Committee included nine urologists, one reproductive endocrinologist, one family physician and one research andrologist. The mission of the Committee 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 committee to produce a comprehensive treatise on male infertility. This document was submitted for peer review by 125 physicians and researchers from the disciplines of urology, gynecology, reproductive endocrinology, primary care and family medicine, andrology and reproductive laboratory medicine. Modifications were made by the Practice Committee of the ASRM. After the final revisions were made based upon the peer review process and the Practice Committee of the ASRM, the documents were submitted to, and approved by the Board of Directors of the AUA and the Board of Directors of the ASRM. These “Best Practice Policies” are intended to assist urologists, gynecologists, reproductive endocrinologists, primary care practitioners and reproductive researchers. Funding of the Committee was provided by the AUA. Committee members received no remuneration for their work. Each member of the Committee provided a conflict of interest disclosure to the AUA.

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