Evaluation of the azoospermic male

Practice Committee of the American Society for Reproductive Medicine in collaboration with the Society for Male Reproduction and Urology

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The purpose of this Technical Bulletin is to review the current methods of diagnosis and strategies for treatment of men with azoospermia. (Fertil Steril® 2008;90:S74–7. ©2008 by American Society for Reproductive Medicine.)

The prevalence of azoospermia is approximately 1% among all men (1) and ranges between 10% and 15% among infertile men (2). Aspermia is distinct from azoospermia and is defined by the complete absence of seminal fluid emission at time of ejaculation. The purpose of this document is to review the current methods of diagnosis and strategies for treatment of men with azoospermia.

INITIAL DIAGNOSIS OF AZOOSPERMIA

The initial diagnosis of azoospermia is established when no spermatozoa can be detected on high-powered microscopic examination of a pellet after centrifugation of the seminal fluid on at least two separate occasions. The World Health Organization recommends that seminal fluid be centrifuged for 15 minutes at 3000 $\times$ *g* or greater (3).

DIFFERENTIAL DIAGNOSIS OF THE AZOOSPERMIC PATIENT

The evaluation of men with azoospermia is aimed at determining the cause and any treatment options that may be effective. The causes of azoospermia can be divided into three main categories: pretesticular, testicular, and posttesticular. Pretesticular causes of azoospermia include endocrine abnormalities having adverse effects on spermatogenesis (secondary testicular failure) and are relatively rare. Testicular causes of azoospermia (primary testicular failure) encompass disorders of spermatogenesis intrinsic to the testes. Posttesticular causes of azoospermia relate to ejaculatory dysfunction or ductal obstructions that prevent sperm from reaching the urethral meatus and can be identified in approximately 40% of affected men (2). Whereas the pretesticular and post-testicular causes of azoospermia frequently are correctable, the testicular causes of azoospermia generally are not; impaired spermatogenesis associated with a varicocele is one possible exception.

INITIAL EVALUATION OF THE AZOOSPERMIC PATIENT

In azoospermic men, the minimum initial evaluation should include a complete medical history, physical examination, and measurement of selected hormones. Relevant medical history includes [1] prior fertility; [2] childhood illnesses and disorders such as viral orchitis or cryptorchidism; [3] genital trauma or pelvic or inguinal surgery; [4] infections such as epididymitis or urethritis; [5] exposure to gonadotoxins such as radiation or chemotherapy, recent fevers or heat exposures, and current or recent medications; and [6] family history of birth defects, mental retardation, reproductive failure, or cystic fibrosis. Physical examination should note: [1] presence of inguinal or scrotal scars; [2] testis size (normal volume >19 mL) and consistency; [3] secondary sex characteristics including body habitus, hair distribution, and gynecomastia; [4] presence and consistency of the vasa deferentia; [5] consistency of the epididymes; [6] presence of varicoceles; and [7] masses palpable on digital rectal examination. The initial endocrine evaluation should include measurements of serum total testosterone and follicle-stimulating hormone (FSH).

EVALUATION OF SPECIFIC CONDITIONS ASSOCIATED WITH AZOOSPERMIA

Results of the initial evaluation direct any further evaluation that may be required to determine the cause of azoospermia. Several specific conditions are associated with azoospermia.

Absence of the Vasa Deferentia (Vasal Agenesis)

Because normal vasa can be palpated easily within the scrotum, the diagnosis of unilateral or bilateral vasal agenesis is made by physical examination. Imaging studies and surgical exploration generally are unnecessary for diagnosis but may help to identify other abnormalities associated with vasal agenesis. Approximately 25% of men with unilateral vasal agenesis and about 10% with congenital bilateral absence of the vasa deferentia (CBAVD) also have unilateral renal agenesis that may be identified by abdominal ultrasonography (4). In azoospermic men with unilateral vasal agenesis, transrectal ultrasonography (TRUS) may help to demonstrate an associated contralateral segmental atresia of the vas deferens or seminal vesicle (5). Due to the embryologic association between the vasa and seminal vesicles, most men with vasal agenesis also have seminal vesicle hypoplasia or agenesis, and because the majority of the seminal fluid derives from the seminal vesicles, almost all men with CBAVD have low semen volume and pH.

There is a strong association between CBAVD and mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene (6, 7). Almost all men with clinical cystic

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fibrosis have CBAVD. Conversely, at least two-thirds of men with CBAVD have mutations of the CFTR gene. However, failure to identify a CFTR abnormality in a man with CBAVD does not exclude a mutation entirely, because many are undetectable using the methods in routine use. In fact, a man with CBAVD should be assumed to harbor a CFTR mutation. Moreover, before any treatments using his sperm, testing should be offered to his female partner to exclude the possibility (approximately 4%) that she too may be a carrier. Ideally, genetic counseling should be offered both before and after genetic testing of both partners. Most men with CBAVD have normal spermatogenesis, but other potential coexisting causes of impaired spermatogenesis should be investigated before harvesting sperm for in vitro fertilization (IVF) (8).

Bilateral Testicular Atrophy

Bilateral testicular atrophy may be caused by either primary or secondary testicular failure. When accompanied by low serum testosterone levels, semen volume also often is low. The results of the initial endocrine evaluation help to distinguish the two possibilities. An elevated serum FSH level and a normal or low serum testosterone concentration imply primary testicular failure. Men with such findings should be offered genetic testing to exclude chromosomal abnormalities and Y-chromosome microdeletions; genetic testing for men with azoospermia is discussed separately in detail in this bulletin. Low serum FSH and testosterone levels and bilateral small testes suggest hypogonadotropic hypogonadism; serum luteinizing hormone (LH) levels usually also are low. Hypogonadotropic hypogonadism results from hypothalamic disorders such as Kallmann syndrome or from congenital or acquired pituitary disorders, including both functional and nonfunctional tumors. Consequently, men with hypogonadotropic hypogonadism merit further evaluation, including measurement of serum prolactin and hypothalamic-pituitary imaging.

Ductal Obstruction

In the absence of vasal agenesis or testicular atrophy, semen volume and serum FSH are key factors in determining the cause of azoospermia. Azoospermic men with normal ejaculate volume may have either a ductal obstruction or an abnormality of spermatogenesis. Azoospermic men with low semen volume and normal sized testes may have ejaculatory dysfunction or ejaculatory duct obstruction.

Men with Normal Ejaculate Volume

In men with normal semen volume, the serum FSH is an important factor for determining whether a diagnostic testicular biopsy may be needed to assess spermatogenesis (9). Marked elevation of serum FSH (greater than two times the upper limit of normal) is a reliable indicator of abnormal spermatogenesis, and, when found, diagnostic testicular biopsy generally is unnecessary. When sperm retrieval for intracytoplasmic sperm injection (ICSI) is considered, a testicular biopsy may be performed for prognostic purposes to determine whether spermatozoa are likely to be retrieved via testicular aspiration or extraction. However, the presence or absence of sperm in a biopsy specimen does not predict absolutely whether sperm are present elsewhere within that testicle; consequently, many experts forego biopsy under such circumstances. Therefore, there is no established consensus of expert opinion regarding the clinical value or utility of prognostic biopsy in men with markedly elevated serum FSH levels.

In contrast, diagnostic testicular biopsy may be indicated for men having a normal serum FSH concentration because the finding does not guarantee normal spermatogenesis. A unilateral or bilateral testicular biopsy is acceptable for such men; there is currently no clear consensus of opinion on the issue. However, a unilateral biopsy should be performed on the larger testis.

Testicular biopsy can be performed using a standard open incision technique or by percutaneous methods. Open testicular biopsy performed under local anesthesia is the most common. A small scrotal incision that does not deliver the testis outside the skin or tunica vaginalis minimizes postoperative scarring and 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. A portion of the testicular tissue obtained may be cryopreserved for future use for ICSI and avoids the need for a second procedure.

A normal testicular biopsy implies obstruction at some level in the reproductive system, and the location must then be determined. Most men with obstructive azoospermia that cannot be attributed to iatrogenic vasal injury have bilateral epididymal obstruction, which can be confirmed only by surgical exploration. Vasography may help to identify obstruction in the vas deferens or ejaculatory ducts. However, due to the risk for vasal scarring and obstruction, vasography should not be performed at the time of diagnostic testicular biopsy unless reconstructive surgery is performed at the same time.

Men with Low Ejaculate Volume

Low ejaculate volume (<1.0 mL) not related to hypogonadism or CBAVD (see previous sections) can be caused by ejaculatory dysfunction, but the most likely cause is ejaculatory duct obstruction (EDO). Although well recognized as a cause of aspermia or low ejaculate volume and oligospermia, ejaculatory dysfunction rarely, if ever, results in low ejaculate volume and azoospermia. Additional seminal parameters that can help to identify EDO are seminal pH and fructose, because seminal vesicle secretions are alkaline and contain fructose. However, semen pH and fructose testing may be misleading when not properly performed, and many experts therefore tend to rely more on other clinical findings.

For the diagnosis of EDO, TRUS is indicated in men with low ejaculate volume, but only rarely in those with normal

volume ejaculates. Whereas vasography is an alternative diagnostic method, TRUS is minimally invasive and avoids the risk of vasal injury associated with vasography (10). Midline cysts, dilated ejaculatory ducts, and/or dilated seminal vesicles (greater than 1.5 cm in anteroposterior diameter) suggest, but do not establish, the diagnosis of EDO (11, 12). Conversely, normal seminal vesicle size does not exclude entirely the possibility of obstruction.

Seminal vesicle aspiration (SVA) and seminal vesiculography may be performed under TRUS guidance and may help to establish the diagnosis of EDO (13). In azoospermic men, the presence of large numbers of sperm in the seminal vesicle strongly suggests EDO. When performed concurrently with SVA, seminal vesiculography can determine the anatomic site of the obstruction. An alternative approach to diagnosis of EDO in azoospermic men with low ejaculate volume involves vasography, simultaneous examination of intravasal fluid for sperm, and testicular biopsy. Success rates achieved with transurethral resection of ejaculatory ducts (TURED) correlate with the cause of the obstruction; midline cysts are the most amenable to surgical treatment (14).

Genetic Testing in Patients with Azoospermia

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

Karyotypic Chromosomal Abnormalities

Chromosomal abnormalities can be identified by karyotype of peripheral leukocytes in approximately 7% of infertile men. The prevalence of such abnormalities relates inversely to the sperm concentration; the prevalence is 10% to 15% in azoospermic men, approximately 5% in oligospermic men, and less than 1% in men having a normal sperm concentration (15, 16). Sex chromosomal aneuploidy (Klinefelter syndrome) accounts for approximately two-thirds of chromosomal abnormalities observed in infertile men. The prevalence of structural abnormalities in the autosomes, such as inversions and translocations, also is higher in infertile men than in the general population. Gross karyotypic abnormalities confer an increased risk for miscarriages and for having children with chromosomal and congenital defects. Therefore, men with nonobstructive azoospermia or severe oligospermia should be karyotyped before their sperm are used for ICSI.

Y-Chromosome Microdeletions

Microdeletions of the Y chromosome may be found in 10% to 15% of men with azoospermia or severe oligospermia (17). Such microdeletions are too small to be detected by karyotyping but can be identified 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 nonoverlapping regions of the long arm of the Y chromosome (Yq11), designated as AZFa (proximal), AZFb (central), and AZFc (distal). These regions of the Y chromosome appear to contain multiple genes necessary for spermatogenesis. For example, the DAZ (deleted in azoospermia) gene, which encodes a transcription factor usually present in men with normal fertility, is located in the AZFc region.

The impact of Y-chromosome microdeletions on spermatogenesis relates to their specific location along the Y chromosome. In men with deletions in the AZFc region, sperm can be present in the ejaculate, albeit in severely reduced numbers. Others with AZFc region deletions will be azoospermic but still may have sufficient sperm production to allow sperm extraction by testis biopsy; in such men, sperm production appears to be stable over time, and the results achieved with ICSI are not affected adversely (18). However, deletions involving the entire AZFb region appear to predict a very poor prognosis for sperm retrieval, even with extensive testicular biopsies (19). The same is true for men with deletions involving the entire AZFa region (20, 21).

Sons of men with a Y-chromosome microdeletion will inherit the abnormality and thus also may be infertile (18, 22). Although Y-chromosome microdeletions are not known to be associated with other health problems, data regarding the phenotypes of sons of men with such abnormalities are still quite limited. A negative test for Y-chromosome microdeletions does not necessarily exclude a genetic abnormality because other still unrecognized gene sequences on the Y or other chromosomes also may be necessary for normal spermatogenesis. Conversely, Y-chromosome microdeletions have been observed in fertile or subfertile men who have fathered children (17, 23).

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. Men with nonobstructive azoospermia should receive genetic counseling and should be offered karyotyping and Y-chromosome analysis before their sperm are used to perform ICSI.

SUMMARY AND RECOMMENDATIONS

- The diagnosis of azoospermia is established when no sperm are detected in at least two separate centrifuged semen samples.
- The minimum initial evaluation of azoospermic men should include a complete medical history, physical examination, and measurements of serum total testosterone and FSH.
- A man with CBAVD should be assumed to harbor a CFTR mutation. Therefore, before any treatments using his sperm, testing should be offered to the female partner to exclude the possibility (approximately 4%)

that she too may be a carrier. All such couples should be offered genetic counseling.

- Men with azoospermia relating to testicular failure should be offered genetic testing to exclude chromosomal abnormalities and Y-chromosome microdeletions. Men with hypogonadotropic hypogonadism should be evaluated by measurement of serum prolactin and hypothalamic-pituitary imaging to exclude both functioning and nonfunctioning pituitary tumors.
- To distinguish between obstructive and nonobstructive causes of azoospermia, diagnostic testicular biopsy may be indicated for men having normal sized testicles, at least one palpable vas deferens, and a normal serum FSH concentration. Vasography should not be performed at the time of diagnostic testicular biopsy unless reconstructive surgery is performed at the same time.
- In azoopermic men with low ejaculate volume and palpable vasa, testicular biopsy may be performed to confirm the presence of reproductive tract obstruction. Transrectal ultrasonography, with or without seminal 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 site of obstruction, but only when reconstructive surgery is performed at the same time.
- Men with nonobstructive azoospermia or severe oligospermia should be informed of the potential associated genetic abnormalities.

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