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 viral orchitis or cryptorchidism; 3) genital trauma or prior pelvic or inguinal surgery; 4) infections such as epididymitis or urethritis; 5) gonadotoxin exposures such as prior radiation therapy/chemotherapy, recent fever or heat exposure and current medications; and 6) family history of birth defects, mental retardation, reproductive failure or cystic fibrosis. Physical examination should note: 1) testis size (normal testis volume greater than 19 ml) and consistency; 2) secondary sex characteristics including body habitus, hair distribution and gynecomastia; 3) presence of and consistency of the vasa deferentia; 4) consistency of the epididymides; 5) presence of a varicocele; and 6) masses upon digital rectal examination. The initial hormonal evaluation should include measurement of serum testosterone and follicle stimulating hormone (FSH) levels.

Recommendation: *The minimum initial evaluation of an azoospermic patient should include a full medical history,*

physical examination, and measurement of serum testosterone and FSH levels.

EVALUATION OF SPECIFIC CONDITIONS ASSOCIATED WITH AZOOSPERMIA

The results of the initial evaluation will direct the strategy that must be used in order to determine the cause of the azoospermia. The following sections discuss the evaluation of several specific conditions associated with azoospermia.

Absence of the Vasa Deferentia (Vasal Agenesis)

Since normal vasa are easily palpable within the scrotum, the diagnosis of vasal agenesis, either bilateral or unilateral, is made 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. For example, an abdominal ultrasound should be considered to rule out renal anomalies. About 25 percent of men with unilateral vasal agenesis and 10 percent of men with congenital bilateral absence of the vasa deferentia (CBAVD) have unilateral renal agenesis (5). In addition, 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 (6). Due to the embryological association between the vasa and seminal vesicles, 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.

There is a strong association between CBAVD and mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene (7, 8). Almost all male patients with clinical cystic fibrosis have CBAVD. Conversely, approximately two-thirds of men with CBAVD have mutations of the CFTR gene. 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 a man with CBAVD harbors a genetic abnormality in the CFTR gene, it is important to test his partner for CFTR gene abnormalities prior to performing a treatment that utilizes his sperm because of the (approximately 4%) risk that she may be a carrier. Ideally, genetic counseling should be offered both before and after genetic testing of both partners.

Recommendations: *At a minimum, genetic testing for CFTR mutations in the female partner should be offered before proceeding with treatments that utilize the sperm of a man with CBAVD. If the female partner tests positive for a CFTR mutation, the male should be tested as well. If the female partner has a negative test for CFTR mutations, testing of the male partner is optional.*

Bilateral Testicular Atrophy

When accompanied by low serum testosterone levels, bilateral testicular atrophy is often associated with low semen volume. Bilateral testicular atrophy 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 either a normal or 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 obstruction of the reproductive system or abnormalities of spermatogenesis. Azoospermic patients with low semen volume and normal sized testes may have ejaculatory dysfunction 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 testicular 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 diagnostic 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 determine whether spermatozoa 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 unilateral 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 postoperative 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 ejaculatory 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 obstructive and nonobstructive causes of azoospermia, diagnostic 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 performed 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 dysfunction 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 seminal parameters that can be helpful in determining the presence of EDO are seminal pH and fructose, since the seminal vesicle secretions are alkaline and contain fructose. However, 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 vesicles (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 determine 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 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 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.

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APPENDIX

This report is part of a series on male infertility. Other titles include: *Report on Optimal Evaluation of the Infertile Male*, *Report on Management of Obstructive Azoospermia*, and *Report on Varicocele and Infertility*.

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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