August 1999

AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE

PRACTICE COMMITTEE REPORT

A Technical Bulletin

NEW TECHNIQUES FOR SPERM ACQUISITION IN OBSTRUCTIVE AZOOSPERMIA

PURPOSE

This guideline describes clinically appropriate methods for retrieval of sperm from men with obstructive azoospermia. Retrieved sperm may then provide an opportunity for fatherhood with the help of assisted reproductive technologies (ART).

PHYSIOLOGY/CLINICAL BASIS

Over the past decade major advances have occurred in the treatment of male infertility. Two developments have been instrumental in the rapid advances made in this area. First, micromanipulation techniques allow severely impaired sperm to fertilize. Secondly, sperm from the testis and epididymis have been used to achieve successful fertilizations with assisted reproduction. These developments have stimulated a renewed interest in techniques of sperm retrieval in obstructive azoospermia. Although the ideal method of sperm retrieval is debatable, the optimal method is that which is safe, efficient, and reliable in retrieving adequate amounts of sperm of optimal quality.

INDICATIONS

Azoospermia may have obstructive and non-obstructive causes. This guideline presents techniques that can be used to treat individuals with obstructive azoospermia. Individuals pursuing these techniques must be familiar with the anatomy, have surgical expertise in the area, and be able to deal with the complications.

Perhaps the best known congenital etiology of male reproductive tract obstruction is congenital bilateral absence of the vas deferens (CBAVD), which occurs in 1.4% of azoospermic patients.[1] This entity has been recently associated with mutations in a gene responsible for cystic fibrosis. Mutations in a gene, known as the cystic fibrosis transmembrane-conductance regulator (CFTR), have been detected in 38 to 71% of individuals with CBAVD.[2,3,4] Abnormalities associated with CBAVD include partial or complete absence of seminal vesicles and epididymis, and renal collecting system anomalies.

Acquired reproductive tract obstruction occurs as a result of infection, vasectomy, or trauma (including iatrogenic injury during bladder neck, pelvic, abdominal, or inguino-scrotal surgery). In general, the optimal treatment would allow the couple to conceive naturally. When this is impossible (as in CBAVD), unlikely to succeed, or has already failed (as in previous attempts at vasovasostomy or vasoepididymostomy) sperm must be retrieved directly from the epididymis, or testis, and combined with ART. With the use of ART, sperm retrieval techniques can result in pregnancy in 24 to 82% of retrievals.[5,6,7-11]

Patient Evaluation

The evaluation for male factor infertility should begin with a thorough history of previous fertility and risk factors for obstruction including inguinal, pelvic, or abdominal surgery, and any history of genitourinary infection. The physical examination should include a careful inspection of the abdomen, inguinal region, and genitalia for surgical scars or signs of trauma. The vas and epididymis should be examined for evidence of obstruction of the epididymis (such as epididymal induration or fullness), and partial or complete absence of the vas deferens and epididymis. A careful digital rectal examination could reveal cystic dilation of the seminal vesicles (often associated with other Wolffian duct anomalies) or a midline prostatic cyst, which can cause ejaculatory duct obstruction.

Laboratory evaluation in these individuals with suspected obstructive azoospermia should include at least one semen analysis and serum for hormone levels. A baseline testosterone level as well as follicle stimulating hormone (FSH) level is necessary. For example, an elevated FSH to three times normal particularly in the presence of small soft testes is virtually diagnostic of primary testicular failure. These blood tests should be evaluated in conjunction with a thorough physical examination since reproductive tract obstruction and impaired spermatogenesis are not mutually exclusive. For many patients with presumptive obstructive azoospermia and normal FSH levels, a testicular biopsy may be required to evaluate sperm production. However, in men with CBAVD who have a normal FSH and normal volume testes, biopsy is not necessary since adequate spermatogenesis can be expected.

For the patient who is a candidate for sperm retrieval, semen analysis should reveal azoospermia or necrospermia; however, other criteria measured in the semen analysis are useful. The volume measurement can distinguish epididymal or vasal obstruction (normal semen volume) from ejaculatory duct obstruction (low volume). The presence of fructose in the specimen as well as an alkaline pH establish that seminal vesicles are present and localize the obstruction to a site(s) proximal to the junction of the vas deferens and seminal vesicle. In addition, the presence of any mature sperm in the semen rules out the possibility of a complete absence of spermatogenesis and suggest improved results from assisted reproductive techniques over that achieved for men with non-obstructive azoospermia.

Genetic testing of men with CBAVD as well as men with idiopathic epididymal obstruction is generally recommended since many of these men have CFTR mutations. Genetic testing of the female partner for CFTR should be performed before any sperm retrieval procedures because not all mutations are identifiable at routine screenings. A negative CF test for the female partner decreases the risk of having a child with cystic fibrosis or CBAVD to less than 1 in 100.

Ultrasound is another important diagnostic and therapeutic tool in the evaluation of infertile, azoospermic men. Renal ultrasound should be obtained in any individual with unilateral or bilateral vasal agenesis due to the common association of renal anomalies with these entities. Transrectal ultrasound (TRUS) can diagnose ejaculatory duct obstruction as well as an obstructing Mullerian or ejaculatory duct cyst.

Contraindications to Sperm Retrieval

In the past, sperm retrieval techniques were reserved for individuals with obstructive azoospermia. Non-obstructive azoospermia, that is, abnormal testicular histology such as maturation arrest or Sertoli cell only, were considered contraindi-

cations to sperm retrieval. Repeated sampling of the testes in non-obstructed azoospermic men, however, has revealed focal spermatogenesis in some men with occasional fully formed sperm.[12] These sperm have been retrieved and used successfully in combination with IVF/ICSI to achieve clinical pregnancies.[13,14,15] Thus non-obstructive azoospermia is no longer an absolute contraindication to sperm retrieval, although sperm retrieval directly from the testis is required. Evaluation and treatment for men with non-obstructive azoospermia are very different from that for men with obstructive azoospermia and are not discussed in this Technical Bulletin.

As previously mentioned, genetic testing of men with CBAVD is recommended since CFTR mutations are found in up to 71%.[2,3,4] Although not an absolute contraindication to sperm retrieval, the detection of CFTR mutations in one or both partners needs to be discussed thoroughly with the couple by a qualified genetic counselor before proceeding with sperm retrieval and assisted reproduction, so that the couple has a complete understanding of their alternatives and the risks of their offspring manifesting or being a carrier for cystic fibrosis.

Physicians should recommend screening for HIV, VDRL, and hepatitis status prior to sperm retrieval and assisted reproduction. Positive tests are an indication for additional evaluation, treatment, and possible counseling before consideration of sperm retrieval and assisted reproduction. Indications and contraindications to sperm retrieval are listed in Table 1.

SPERM RETRIEVAL TECHNIQUES

Sperm retrieval should be considered for men at the time of difficult surgical reconstruction, as well as in individuals with unreconstructable reproductive tract-obstruction or for men with obstruction who decline to have surgical reconstruction. The goals of sperm retrieval are listed in Table 2. The techniques that are described below include sperm retrieval at the time of surgical reconstruction, microsurgical epididymal sperm aspiration, intraoperative testicular sperm retrieval, and percutaneous techniques of epididymal and testicular sperm retrieval (Table 3).

Intraoperative Testicular Sperm Retrieval During Vasovasostomy and Vasoepididymostomy

Since only 20 to 40% of couples conceive after attempted vasoepididymostomy despite patency rates of 70 to 80%, consideration should be given to sperm retrieval at the time of surgical reconstruction. This is especially important in individuals for whom a difficult reconstruction is anticipated. These individuals include patients who have undergone previous vasovasostomy or vasoepididymostomy or other scrotal procedures where inflammation or scarring may obscure surgical planes. A thorough discussion of the risks, benefits, need for assisted reproductive treatment, and cost of cryopreservation should take place prior to surgical intervention, since not all couples will be willing or able to pursue this option.

If motile sperm are found at the repair site, sperm may be aspirated at the repair site and cryopreserved. Since the most motile and best quality sperm are usually obtained in the most proximal epididymis, and the success of reconstruction is best when repair is most distal; no compromise in repair sites should be made in order to get better sperm for cryopreservation. Retrieving sperm from a proximal tubule may cause proximal obstruction at that site. Instead, sperm may be retrieved from a testicular biopsy. The tunica albuginea is incised and a generous portion of seminiferous tubules is extruded by gentle compression. Sperm may then be extracted from this tissue using a mincing technique, vortexing, or sequential passages of the tes-

ticular tissue suspension through a narrow gauge needle. Care must be taken to avoid injuring testicular blood vessels in the tunica albuginea during biopsy retrievals. Sperm obtained from the testis in this fashion are retrieved in small numbers and have been described as non-motile or "barely twitching." Subsequent incubation in vitro may allow the spermatozoa to acquire some motility. Despite their initial appearance, these sperm are almost always functional, and pregnancies have been achieved with fresh and cryopreserved testicular sperm.

EPIDIDYMAL SPERM RETRIEVAL TECHNIQUES

Microsurgical Epididymal Sperm Aspiration

In cases of CBAVD, unreconstructable reproductive tract obstruction, or in cases where the patient chooses not to have surgical reconstruction, epididymal sperm can be aspirated as an isolated surgical procedure. When performed as an operative microsurgical procedure, these techniques are referred to as microsurgical epididymal sperm aspiration (MESA).

To avoid contamination of sperm with blood cells during aspiration, the technique of micropuncture of the epididymal tubule was developed.[5] Briefly, single epididymal tubules can be identified under the operating microscope and individually aspirated with an atraumatic technique. Sequential micropunctures can be performed until optimal sperm quality has been obtained. Puncture sites are closed or cauterized. An alternative approach is to incise tubules and gather fluid after it flows out of the tubules. Only microliters of fluid need to be retrieved since sperm in the epididymal fluid are highly concentrated (roughly 1 x 10^6 sperm/µl). In this way, MESA provides for more than adequate numbers of sperm for immediate use, as well as for cryopreservation.

Percutaneous Epididymal Sperm Aspiration

Epididymal aspiration can also be performed without surgical scrotal exploration, can be repeated easily and at low cost, and does not require an operating microscope or expertise in microsurgery. Percutaneous epididymal sperm aspiration (PESA) can be performed under local or general anesthesia.[6] After induction of anesthesia, the testis is stabilized and the epididymis is held between the surgeon's thumb and forefinger. A butterfly needle attached to a 20 ml syringe is inserted into the caput epididymis and withdrawn gently until fluid can be seen entering the tubing of the aspiration set. The procedure is repeated until adequate amounts of epididymal fluid are retrieved (see Figure 1). If no sperm are retrieved, then it is recommended to proceed with MESA, testis biopsy, or testicular aspiration.

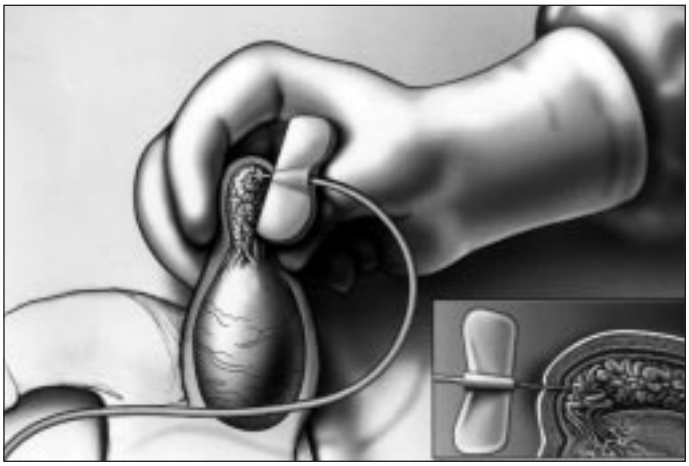

Figure 1. Schematic representation of percutaneous epididymal sperm retrieval. With the testis stabilized between the surgeon's thumb and forefinger, a butterfly needle attached to a syringe is inserted into the caput epididymis and withdrawn until fluid is seen entering the tubing.

TESTICULAR SPERM RETRIEVAL TECHNIQUES

Open Testicular Biopsy

Open testicular biopsies can be used to obtain sperm. This tech-

nique is best applicable to men with non-obstructive azoospermia. The testicular arteries are end arteries and injury during biopsy may cause partial testicular infarction.[16] Microscopic open biopsy may allow sperm to be obtained without injury to blood supply.[17]

Percutaneous Testicular Sperm Aspiration

Several authors who used open or percutaneous methods to retrieve sperm directly from the testis have reported fertilization and subsequent pregnancies with testicular sperm.

The technique of percutaneous fine needle aspiration (FNA) of the testis was initially described as a diagnostic procedure in azoospermic men.[18] This is also sometimes called testicular sperm aspiration or TESA. More recently FNA has been used to retrieve sperm for assisted reproductive techniques (see Figure 2). In this procedure, the testis is stabilized between the surgeon's thumb and forefinger and a needle is inserted along the long axis of the testis. The needle is withdrawn slightly and redirected in order to disrupt the testicular architecture. The procedure is repeated until adequate testicular material has been aspirated.

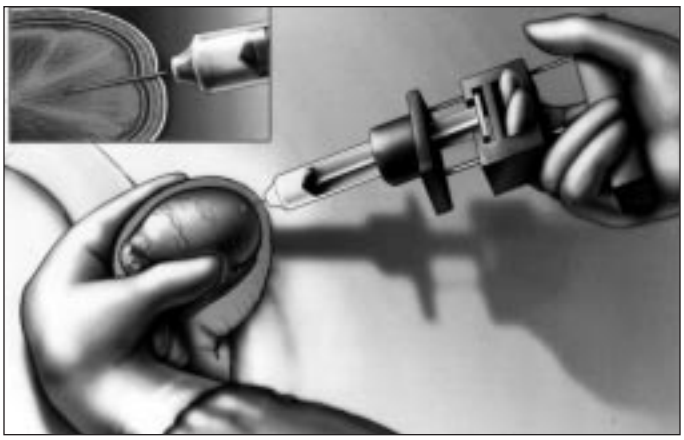

Figure 2. Schematic representation of percutaneous testicular sperm retrieval. The testis is stabilized between the surgeon's thumb and forefinger and the needle is inserted along the long axis of the testis. The needle is withdrawn slightly and redirected in order to disrupt the testicular architecture. The procedure is repeated until adequate testicular material has been aspirated. A Franzen needle holder can be used to provide negative pressure for needle aspiration.

Percutaneous Testicular Biopsy (PercBiopsy)

A technique of percutaneous biopsy of the testis has also been described. A 14-gauge biopsy gun with a short (1 cm) excursion is used to retrieve testicular tissue. Anesthesia is achieved with a spermatic cord block, and multiple biopsies can be obtained through a single entry site. The core needle provides better sperm yield than fine needle aspiration and is relatively simple to use.[19] The advantages and disadvantages of each of the techniques for sperm acquisition are listed in Table 4.

IMPORTANCE OF ASSISTED REPRODUCTION

Once viable spermatozoa have been retrieved, optimal chances to achieve a pregnancy require the application of the assisted reproductive technique of intracytoplasmic sperm injection (ICSI). Although anecdotal pregnancies have occurred after intrauterine insemination with surgically retrieved sperm, reports by several centers comparing fertilization and pregnancy rates in standard IVF versus IVF with ICSI have shown the importance of the assisted reproductive technique used in conjunction with sperm retrieval. The sperm microaspiration retrieval and assisted reproductive technologies (SMART) study[20] reviewed the overall United States experience with microaspiration retrieval and assisted reproductive techniques. The overall clinical pregnancy rate for the 219 retrieval procedures performed was only 10% using microsurgically retrieved sperm and IVF. The results with retrieved sperm used in combination with IVF/ICSI are far superior. Fertilization rates per oocyte injected of 24 to 82% have been achieved utilizing sperm retrieval from azoospermic men. The absence of a correlation between sperm density, motility, or morphology with ejaculated sperm and ICSI outcome has made this the assisted reproductive technique of choice in couples with male factor

infertility.[21,22,23] So dramatic are these improvements in outcome that maternal factors (maternal age, oocyte number, oocyte quality) are now considered to be the principal determinants in ICSI outcome in couples with male factor infertility.

It has been reported that success with ICSI is independent of the three basic semen parameters: sperm concentration, motility, or morphology.[21,22,23] However, studies have correlated maternal age with ICSI outcome using ejaculated sperm.[24,25] Pregnancy rates clearly decline with advanced maternal age (see Table 5). Although few data have been published, it has been observed that pregnancy rates after ICSI with retrieved spermatozoa are highly dependent on maternal age.

IMPORTANCE OF CRYOPRESERVATION

Successful cryopreservation of semen has proved an essential tool in the treatment of infertile couples. Cryopreservation allows for temporal separation of sperm retrieval procedures from assisted reproductive techniques, enables detection of infectious disease prior to insemination, and has enabled men with malignancies requiring chemotherapy to father children.

Prior to the advent of ICSI, fertilization and pregnancy rates achieved with cryopreserved sperm recovered with microsurgery were dismal. The poor results are attributed to the fact that cryopreservation has detrimental effects on sperm motility[26] and sperm acrosomes.[27] Since ICSI enables even severely impaired viable sperm to achieve fertilization, cryopreserved sperm can achieve acceptable rates of fertilization and pregnancy when coupled with ICSI. Whenever available, excess retrieved spermatozoa should be cryopreserved to avoid unnecessary subsequent sperm retrieval procedures.

RISKS OF INTERVENTION

Morbidity

More invasive techniques of sperm retrieval such as MESA involve longer recovery for the man than percutaneous procedures of sperm retrieval from the testis or epididymis. The frequency of hematoma formation and other complications after MESA, PESA, or percutaneous testicular aspiration appears to be low. Since more sperm of higher quality are typically retrieved during a MESA procedure, only one intervention is usually necessary, whereas repeat percutaneous testicular aspirations are typically necessary if the first ICSI procedure does not result in a live birth.

Sperm retrieval techniques appear to be safe, especially when performed under local anesthesia. Proximal sperm retrieval may result in permanent obstruction of the proximal epididymis and prevent subsequent consideration of microsurgical reconstruction. This risk should be considered prior to the choice of sperm retrieval over reconstruction. Permanent testicular devascularization has been reported after attempted sperm retrieval from multiple testicular sites.[28] However, operative morbidity and permanent injury to the testis appear to be rare.

SUMMARY

Sperm retrieval with ART is an alternative to microsurgical repair for men with correctable reproductive tract obstruction. It is the only opportunity for men with irreparable obstruction to have their own genetic children. The ability to use immature (testicular or epididymal) sperm with assisted reproduction is a novel observation. In addition to the nearly constant presence of testicular sperm in men with obstructive azoospermia, men with non-obstructive azoospermia will often have a few sperm pre-

sent in their testes on biopsy.[5] The experience with sperm retrieval and ICSI may now be translated into a chance of fertility for nearly all men, despite severe impairments in spermatogenesis.[29,30] For couples in whom the man has bilateral vasal agenesis, genetic testing of the female partner is critical to evaluate cystic fibrosis gene mutation status. It is important to remember that not all cystic fibrosis (CF) gene mutations are identifiable with routine testing. Therefore, a negative CF evaluation does not completely eliminate the risk of having a child affected by CF or CBAVD. For women with a negative CF screen for at least the 12 most common CF mutations, the risk of having a child affected by CF or CBAVD is less than 1 %.

Extensive experience with the use of sperm retrieved from the obstructed reproductive system and ICSI is not yet available. Although most clinicians consider continued application of these technologies to treat men with obstructive azoospermia acceptable, it is important to note that at least one authority, the Dutch government, has declared a moratorium of MESA/ICSI and TESE/ICSI because of concerns with the use of "aged" sperm.[31] Sperm that have "aged" in an obstructed system may have an increased risk of DNA breaks.[32] Since few children have been born after sperm retrieval/ICSI procedures, this risk of genetic abnormalities is not yet fully evaluable. Initial results suggest that there is no dramatically increased risk of birth detects after sperm retrieval with ICSI.[33]

The best technique for sperm aspiration in obstructive azoospermia has not been determined. The requirement for microsurgical expertise and the longer recovery after an open surgical procedure relative to percutaneous procedures must be considered. For repeat sperm retrieval procedures, up to six months may be necessary to allow complete healing and restoration of optimal spermatogenesis after testicular epididymal sperm retrieval.[28] Recommendations regarding sperm retrieval are listed in Table 6. The recommendations in this Technical Bulletin are based on Grade B evidence according to the ACOG Practice Patterns criteria. All references in this technical bulletin are based on Grade III evidence according to the U.S. Preventive Services Task Force criteria.

Table 1. Indications and relative contraindications to sperm acquisition.

Indications	Relative Contraindications
Obstructive azoospermia	Genetic mutation
Unreconstructable reproductive tract obstruction (RTO)	HIV
Reconstructable RTO requesting sperm retrieval instead of surgical repair	Hepatitis
	Maternal age greater than 42

Table 2. Goals of sperm retrieval procedures

Obtain the best quality sperm possible
Retrieve an adequate number of sperm for both immediate use and for cryopreservation
Minimize damage to the reproductive tract so as not to jeopardize future attempts at sperm retrieval or surgical reconstruction

Table 3. Sperm Retrieval Techniques

	Epididymal	Testicular
Open Biopsy	Epididymal Sperm Aspiration MESA	Open Testicular Biopsy
Percutaneous	Percutaneous Epididymal Sperm Aspiration PESA PESE	Percutaneous Testicular Biopsy PercBiopsy FNA TESA

MESA = Microsurgical epididymal sperm aspiration; TESE = Testicular sperm extraction;
PESA = Percutaneous epididymal sperm aspiration; PercBiopsy = Percutaneous testicular biopsy.

Table 4. Advantages and disadvantages of sperm retrieval techniques.

	Advantages	Disadvantages
MESA	Reduced blood contamination Large number of sperm retrieved Excellent results with cryopreservation Reduced risk of hematoma Best clinical pregnancy rates	Requires microsurgical expertise Increased cost General anesthesia Scrotal exploration required Postoperative discomfort
TESE	No microsurgical expertise required Local or general anesthesia Few instruments Fast and repeatable	Few sperm retrieved Risk of testicular atrophy
PESA	No microsurgical expertise required Local anesthesia Few instruments Fast and repeatable Minimal postoperative discomfort	Few sperm retrieved Risk of hematoma Damage to adjacent tissue Blood contamination
PercBiopsy	No microsurgical expertise required Local anesthesia Few instruments Fast and repeatable Minimal postoperative discomfort	Few sperm retrieved Risk of testicular atrophy Risk of hematoma Blood contamination

MESA = Microsurgical epididymal sperm aspiration; TESE = Testicular sperm extraction;
PESA = Percutaneous epididymal sperm aspiration; PercBiopsy = Percutaneous testicular biopsy.

Table 5. Fertilization and clinical pregnancy rates resulting from ICSI using ejaculated sperm according to maternal age.

Author	Number of cycles	Fertilizations/oocytes injected (%)			Clinical pregnancies/ cycle(%)		
		≤34	35-39	≥40	≤34	35-39	≥40
Oehninger[18]	102	61	62	58	45	22	6
Sherins[19]	229	41	38	31	23	10	10

Table 6. Recommendations regarding sperm retrieval.

1. Regardless of the sperm retrieval technique used, intracytoplasmic sperm injection should be regarded as the assisted reproductive technique of choice due to its superior results compared to IUI or IVF alone in cases of severe male factor infertility.

2. Genetic testing of both partners is recommended in all couples with congenital reproductive tract obstruction due to the association with CFTR abnormalities.

3. All couples undergoing complex reconstruction should consider cryopreservation of sperm due to the incidence of late surgical failures.

4. Whether sperm retrieval is indicated should be considered carefully with genetic mutations, HIV, hepatitis, and maternal age greater than 42.

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This report was developed under the direction of the Practice Committee of the American Society for Reproductive Medicine as a service to its members and other practicing clinicians. While this document reflects appropriate management of a problem encountered in the practice of reproductive medicine, it is not intended to be the only approved standard of practice or to dictate an exclusive course of treatment. Other plans of management may be appropriate, taking into account the needs of the individual patient, available resources, and institutional or clinical practice limitations. The committee wishes to thank Peter N. Schlegel, M.D., and Sarah K. Girardi, M.D., for their assistance in preparing this Technical Bulletin.

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