Contemporary OB/GYN® Archive
April 15, 1997

Sperm retrieval for assisted reproductive technologies

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Ejaculated or surgically retrieved spermatozoa, including immature sperm retrieved from the epididymis and testis, may be used for intracytoplasmic sperm injection.

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One in every six married couples in the US will seek medical evaluation for assistance with fertility, and in up to 50% of couples a male factor is identified. The most severe expression of male factor infertility is azoospermia, where no sperm are present in the ejaculate. Causes of azoospermia include congenital and acquired reproductive tract obstruction as well as spermatogenic failure. Less than a decade ago, patients with azoospermia were often unable to be successfully treated. Since the introduction of intracytoplasmic sperm injection, treatment of most men with azoospermia can now be considered for treatment, even if the azoospermia is caused by testicular failure. [1] [2]

Intracytoplasmic sperm injection (ICSI), a technique performed as part of an in vitro fertilization (IVF) cycle, has revolutionized the treatment of severe male factor infertility. ICSI involves the injection of a single sperm into each oocyte, in vitro, during an IVF cycle. ICSI essentially bypasses all natural barriers to fertilization, such as sperm interaction with the zona pellucida and sperm-egg fusion. As long as there is sperm viability, fertilization rates with ICSI will be comparable with those achieved during IVF with normal spermatozoa. Subsequent pregnancy rates then depend primarily on female factors, emphasizing the tremendous value of ICSI in overriding specific sperm defects that heretofore may have limited treatment of severe male factor infertility.

Evaluation prior to sperm retrieval

Before initiating treatment for a couple in whom the man has azoospermia, it is important to determine whether the lack of sperm in the ejaculate is from an obstructive or nonobstructive process. Careful evaluation of the postejaculate urinalysis is also necessary to rule out retrograde ejaculation. Functional ejaculatory abnormalities should be ruled out as well. Men with retrograde ejaculation do not require invasive procedures for sperm retrieval; sperm may be isolated from urine or catheterized from the bladder and used for intrauterine insemination or IVF. Men with functional ejaculatory disturbances may be treated with behavioral counseling, vibratory stimulation, or electroejaculation and do not require surgical sperm retrieval.

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History and physical examination can suggest the cause of azoospermia for an experienced examiner. For example, the presence of normal volume testes with bilaterally indurated epididymides or absent vasa deferentia will point to an obstructive cause of azoospermia. A history of cryptorchidism in the presence of small volume or soft testes may suggest nonobstructive azoospermia, especially if supported by an elevated serum follicle-stimulating hormone (FSH) level. In most cases, bilateral testicular biopsy will be indicated to confirm the impression of obstructive or nonobstructive azoospermia.

The distinction between obstructive and nonobstructive azoospermia is not of academic interest alone. For men interested in restoring fertility after vasectomy, microsurgical reconstruction may be a better option than proceeding directly to assisted reproduction. 3 Men with obstructive azoospermia have a higher success rate for sperm retrieval and are at risk of different genetic defects than men with nonobstructive azoospermia. Because the success rate and genetic risks to offspring may determine whether a couple will proceed with sperm retrieval and ICSI, it is worthwhile to make this determination before treatment.

Bilateral vasal agenesis is the most common congenital anomaly of the male reproductive tract that causes obstructive azoospermia. Congenital bilateral absence of the vas deferens is found in 1% to 2% of the infertile male population, and 42% to 66% of men with this defect will have one or more cystic fibrosis gene mutations detectable. Conversely, almost all men with cystic fibrosis manifest infertility because of bilaterally absent vasa. Also, up to 47% of men with idiopathic epididymal obstruction are known to be carriers for cystic fibrosis gene mutations. 4 It is critically important to screen the partners of men with vasalogenesis or with idiopathic epididymal obstruction. Cystic fibrosis gene mutation testing of the woman should be done prior to attempts at conception with sperm retrieval and assisted reproduction, since not all of the man's CF mutations may be detected with routine testing.

For men with nonobstructive azoospermia, different genetic defects should be considered. Up to 13% or more men with this condition will have microdeletions of the Y chromosome, including deletions of the DAZ (deleted in azoospermia) gene. 5 Preliminary evidence suggests that additional men with severe male factor infertility may have germ cell defects of the Y chromosome. 6 Other chromosomal abnormalities, detectable on karyotype analysis, are also frequently found on evaluation of these men, and may be transmitted to offspring by ICSI procedures. When genetic abnormalities are found, the couple should be referred for genetic counseling prior to attempts at assisted conception. Since some attempts at sperm retrieval are not successful for men with nonobstructive azoospermia, the potential for use of donor spermatozoa should be raised with the couple. In many cases, the couple is ambivalent regarding this option and psychological counseling may be helpful to review the implications of having a child who is not genetically related to the father.

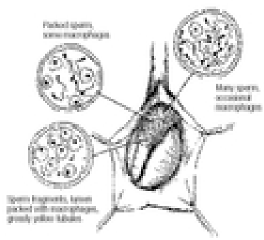
Sperm quality in obstructive and nonobstructive azoospermia

In the normal male reproductive tract, sperm exiting the testis have minimal motility and limited egg fertilizing capacity. Sperm acquire the potential for improved motility and fertilizing ability during epididymal transit. So, in the unobstructed epididymis, sperm of optimal quality (as evaluated by percent motile cells) are found in the most distal portion. The obstructed epididymis shows the opposite pattern, with sperm of optimal quality in the proximal portion and sperm of very poor quality in the most distal segments (Figure 1). This finding of "inverted motility" is expected in the obstructed male reproductive tract since sperm production continues in the testis and reabsorption of those sperm is an active process in the most distal regions of the system. The most distal obstructed epididymis tends to contain dilated yellow tubules that are packed with macrophages reabsorbing old, degenerated sperm. Therefore, sperm retrieval should be performed from the proximal obstructed epididymis.

FIGURE 1

This schematic shows the intraluminal contents of the chronically obstructed epididymis in congenital absence of the vas deferens. Unlike the unobstructed system, better-quality sperm can be found proximally in the chronically obstructed system, whereas degenerating sperm and macrophages filled with sperm remnants are found distally.

Source: Schlegel PN, et al: Fertil Steril; 1994;61:895-901.



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In nonobstructive azoospermia (testicular failure), some sperm production is at least focally present within the testis, despite the fact that inadequate numbers of sperm are released from the testis to contribute to the ejaculate. 7 A common observation for testicular sperm samples is that retrieved spermatozoa are not motile or have a sluggish twitching motion. After several hours of incubation in vitro, testicular sperm typically show some motility. The lack of initial motility does not necessarily reflect a lack of viability for testicular sperm, since these sperm have never acquired motility. In distinction, nonmotile ejaculated sperm have acquired and lost motility as sperm viability is lost, rendering the sperm useless for ICSI.

Typical criteria for a poor prognosis of sperm production, including an elevated serum FSH level or decreased testicular volume, do not prospectively predict which patients can have sperm found with testicular sperm extraction (TESE). Even the histologic patterns on diagnostic testicular biopsy (Sertoli cell-only, maturation arrest, hypospermatogenesis) cannot definitively predict the chance of finding sperm with TESE. However, many centers require diagnostic biopsies prior to TESE procedures to rule out carcinoma in situ (intratubular germ cell neoplasia) that is present in up to 3% of men with nonobstructive azoospermia who are candidates for treatment with TESE-ICSI.

Sperm retrieval techniques

There are three goals of sperm retrieval: to obtain the best-quality sperm possible, to retrieve an adequate number of sperm for both immediate use and cryopreservation (where possible), and to minimize damage to the reproductive tract so as not to jeopardize future attempts at sperm retrieval or testicular function. For men with nonobstructive azoospermia, sperm retrieval has most commonly been performed with an open extraction procedure that allows sampling of large volumes of testicular parenchyma, since the concentration of sperm is so low in the testis.

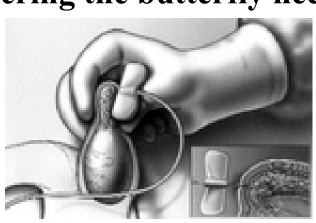
Testicular sperm extraction (TESE). Testicular sperm retrieval is usually timed to coincide with oocyte retrieval during a planned IVF cycle. Under local or general anesthesia, sperm retrieval is effected using an open testicular biopsy technique. Direct identification of testicular blood vessels is achieved using optical magnification with loupes or an operating microscope. Blood supply to the testis travels in a serpiginous fashion under the capsule (tunica albuginea) of the testis. Retrieval of ample amounts of testicular parenchyma can be achieved through an avascular region in the tunica albuginea near the midportion of the medial or lateral testicular surface with a single large incision in the tunica albuginea. The testicular parenchyma (approximately 500 mg) is excised with sharp, curved iris scissors and placed in medium supplemented with plasma protein fraction (Plasmanate). 8

Immediate dispersal of seminiferous tubules is mechanically achieved by separating individual tubules, cutting them with scissors or performing sequential passes of tissue suspension through a 24-gauge angiocatheter. Sequential samples of testis parenchyma are sampled and examined in the operating room with phase contrast microscopy at 400 * power. Sampling is continued until spermatozoa are found or it is felt to be unsafe to perform additional biopsies. The incised segment of tunica albuginea is closed. Biopsy of the contralateral testis is performed if no sperm have been found. Multiple incisional biopsies should be avoided during TESE to prevent devascularization of the testis from damage to the subtunical testicular blood supply. It is also important to remember that sperm production is marginal in men with nonobstructive azoospermia; local inflammation and recovery after TESE may adversely affect sperm production for up to 6 months. Therefore, at least 6 months should elapse between TESE procedures. 9 For men with obstructive azoospermia, a variety of techniques for sperm retrieval have been successfully applied.

Percutaneous epididymal sperm aspiration (PESA). PESA has been advocated as superior to open microsurgical approaches in that it can be performed without surgical scrotal exploration, it is repeated easily at low cost, and it does not require an operating microscope or expertise in microsurgery. The procedure can be performed under local or general anesthesia. 11 The testis is stabilized and the epididymis is held between the surgeon's thumb and forefinger. A 21-gauge butterfly needle attached to a 20-mL syringe is inserted into the caput epididymis and withdrawn gently until fluid can be seen entering the butterfly needle tubing (Figure 2). The procedure is repeated until adequate numbers of sperm are retrieved. PESA yields very small amounts of epididymal fluid and contamination with blood cells is frequent. For the 10% to 20% of attempts where sperm are not retrieved with PESA, open sperm retrieval or percutaneous testicular aspiration are possible.

FIGURE 2

With percutaneous epididymal sperm aspiration (PESA), the testis is stabilized and the epididymis is held between the surgeon's thumb and forefinger. A 21-gauge butterfly needle attached to a 20-mL syringe is inserted into the caput epididymis and withdrawn gently until fluid can be seen entering the butterfly needle tubing.



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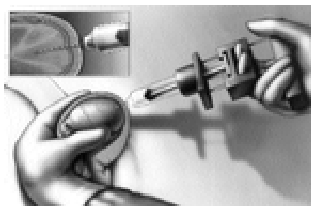
Percutaneous testicular sperm aspiration. The technique of testicular fine-needle aspiration (TFNA) of the testis was initially described as a diagnostic procedure in azoospermic men. Subsequently, testicular fine-needle aspiration or biopsy for the recovery of spermatozoa have been described. 12 Percutaneous puncture and aspiration of the testis can be performed using a 21 to 23-gauge needle connected to a 20-mL syringe in a Franzen syringe holder (Figure 3). Percutaneous testicular biopsy can be performed with an automatic biopsy gun. The limited published experience to date with TFNA makes critical evaluation of this technique difficult, although it is evident from our experience that sperm retrieval is routinely possible with TFNA for men with obstructive azoospermia, and that occasional hematoceles and hematomas may occur after percutaneous sperm retrieval.

FIGURE 3

In this schematic representation of testicular fine needle aspiration (TFNA), the testis is stabilized between the surgeon's thumb and forefinger and a 22-gauge needle is inserted into the testis. Negative pressure is applied, and the needle is withdrawn slightly and redirected in order to disrupt the seminiferous tubules of the testis. The procedure is repeated until adequate testicular fluid has been aspirated. A Franzen syringe holder is used to provide negative pressure during aspiration.



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The advantages of percutaneous aspiration techniques are that they can be performed with less anesthesia, without open scrotal exploration and its attendant postoperative discomfort, and without microsurgical expertise. The percutaneous techniques are easily repeatable as well as potentially less expensive than microsurgical techniques and do not require special microsurgical training.

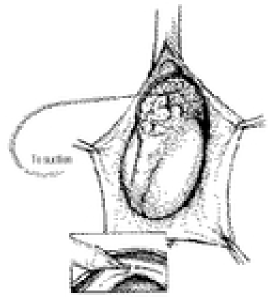
However, a lower pregnancy rate has been observed after percutaneous sperm retrieval (24%) than that achieved with microsurgical epididymal sperm aspiration (36% to 82%) with ICSI. 4 The inconsistent ability to retrieve adequate numbers and quality of sperm for cryopreservation with percutaneous approaches is also a disadvantage compared with open sperm retrieval techniques.

Although fine-needle aspiration has been reported as a technique of sperm retrieval for nonobstructive azoospermia, men have not been adequately characterized with formal testicular biopsy before these procedures, and it is possible that some of the patients were actually obstructed. Fine-needle aspiration would be much more likely to retrieve sperm in men who are obstructed and may make results of treatment unevaluable unless diagnostic biopsies were performed.

Microsugical epididymal sperm aspiration (MESA). Epididymal sperm retrieval was initially performed using an open, microsurgical technique commonly referred to as microsurgical epididymal sperm aspiration (MESA). This technique offers the advantages of dissection of individual epididymal tubules under direct vision, meticulous hemostasis to minimize sperm contamination with blood cells, and aspiration of multiple sites to retrieve large numbers of sperm with optimal quality to allow immediate sperm use for assisted reproduction as well as sperm cryopreservation. To avoid contamination of sperm by blood cells, a technique of micropuncture was developed. 4 Individual tubules are cleanly punctured, as occurs during venipuncture for blood drawing. With gentle aspiration, epididymal fluid passes through the glass micropipet, through the Silastic tubing, and into a plastic tuberculin syringe (Figure 4). Typically, only microliters of fluid need to be retrieved. Sperm in the epididymal fluid are highly concentrated (roughly $1 * 10^6$ sperm/µL) so this technique provides far more than an adequate number of sperm. Since post-thaw sperm viability is related to prefreeze quality, it is worthwhile to retrieve and cryopreserve sperm of optimal quality to avoid the need for additional sperm retrieval procedures.

FIGURE 4

In microsurgical epididymal sperm retrieval for congenital absence of the vas deferens, a single epididymal tubule is punctured with the micropuncture apparatus that includes a glass micropipet, silicone tubing, a three-way stopcock, a 1-mL plastic tuberculin syringe, and a very clean, dry glass syringe for equilibration of pressures.



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Cryopreservation of epididymal and testicular sperm

Successful sperm cryopreservation allows the opportunity for multiple ICSI cycles without the need for additional sperm retrieval procedures. Since ICSI enables even severely impaired sperm to effect oocyte fertilization, as long as sperm are viable, cryopreserved sperm can achieve acceptable rates of fertilization and pregnancy when coupled with ICSI. Cryopreservation also allows the potential for temporal separation of sperm retrieval procedures from assisted reproductive techniques. This is an important benefit for the center that performs only occasional sperm retrieval procedures. If the cryopreserved sperm are viable after thawing, then this approach does not compromise the chance for achieving a pregnancy. Unfortunately, up to 10% to 20% of frozen epididymal samples will not have adequate viability after thawing for use with ICSI. Freezing of isolated spermatozoa from testicular tissue is difficult because of the low number of spermatozoa present in testicular tissue and the limited sperm motility of testicular sperm, which makes documentation of sperm viability difficult. Nevertheless, anecdotal reports have indicated that it is possible to cryopreserve testicular biopsy tissue samples and subsequently extract spermatozoa after thawing with at least isolated pregnancies achieved.

Importance of assisted reproduction

Initial attempts at conception using retrieved spermatozoa were limited by poor fertilization and pregnancy rates with intrauterine insemination or standard IVF. A multicenter US survey of over 100 MESA-IVF cycles in 1994 revealed a less than 10% clinical pregnancy rate per sperm and egg retrieval procedure. The introduction of early assisted fertilization procedures such as subzonal insertion (SuZI) and partial zona dissection (PZD) improved clinical pregnancy rates up to 27% per sperm and egg retrieval procedure. With the introduction of ICSI in 1992, the ability to achieve high fertilization and pregnancy rates with epididymal sperm was realized.

ICSI can be performed only during an IVF cycle, which involves controlled ovarian hyperstimulation, (transvaginal) oocyte retrieval, and embryo transfer after fertilization is achieved in vitro. The immature nature of testicular and epididymal sperm is reflected in limited sperm cell membrane permeability. At least one report suggests that special preparation of immature sperm with aggressive manipulation to effect increased sperm membrane permeability prior to ICSI is necessary to achieve optimal fertilization and pregnancy rates. 13

Results with sperm retrieval

Results with sperm retrieval and IVF/ICSI should optimally be represented as clinical or ongoing/ delivered pregnancy rates per sperm and egg retrieval attempt. Pregnancy rates per transfer or other subsets of data should be avoided, if at all possible, since they do not provide adequate information to a couple about their chances of pregnancy after sperm retrieval procedures.

Obstructive azoospermia. Pregnancy rates after sperm retrieval will depend on the certainty with which the diagnosis of obstruction is made prior to retrieval as well as the overall results achieved at that IVF unit. Pregnancy rates with larger series have ranged from 24% per sperm and egg retrieval procedure with PESA, up to 80% with simultaneous MESA-ICSI and aggressive sperm immobilization. Overall results with sperm retrieval-ICSI for men with obstructive azoospermia are presented in Table 1. Most series have indicated that there are no individual characteristics of men that will further predict the chance of achieving a pregnancy after a sperm retrieval-ICSI procedure. For example, there appears to be no difference in results for men with congenital absence of the vas deferens versus idiopathic epididymal obstruction or for men postvasectomy. There does appear to be a difference between results for obstructive and nonobstructive azoospermia, in part because not all men with nonobstructive azoospermia have sperm retrievable with TESE.

TABLE 1: Results of ICSI with testicular and epididymal sperm in the treatment of obstructive azoospermia

Study	Clinical pregnancy rates	Sperm retrieval technique
Palermo et al.[18]	14/17 (82%)	MESA
Schlegel et al.[19]	14/27 (48%)	MESA
Silber et al.[16]	8/17 (47%)	MESA
Belker et al.[17]	6/15 (40%)	TFNA
Gil-Salom et al.[20]	4/15 (27%)	Testicular biopsy
Craft et al.[11]	10/42 (24%)	PESA

MESA-microsurgical epididymal sperm aspiration; PESA-percutaneous epididymal sperm aspiration; TFNA-testicular fine-needle aspiration

Nonobstructive azoospermia. Results of sperm retrieval (TESE)-ICSI for men with nonobstructive azoospermia have two different components. The first is the ability to retrieve sperm from the testis, and the second is the pregnancy rate after ICSI using those sperm. The chance of retrieving sperm has ranged up to 90% for men without obstruction in a small series and as low as 50% in larger series. Obviously, the more carefully men are selected before retrieval will determine the likelihood of finding sperm with TESE. One team initially reported that 12 (80%) of 15 men had sperm retrieved with TESE. The fertilization rate per injected oocyte was 48%, with an overall clinical pregnancy rate of 20% (3/15) per attempt at TESE. [14] Subsequent results from Belgium suggest that the rate of sperm retrieval is closer to 50% per TESE procedure. Another group reported that 10/16 (62%) of men without obstruction had sperm extracted with TESE. As Table 2 shows, the fertilization rate per injected oocyte was 58%, and 5/10 (50%) of those undergoing ICSI achieved a clinical pregnancy (5/16 overall; (31% per TESE attempt)). [8]

TABLE 2: Fertilization and pregnancy rate for

testicular sperm retrieval-ICSI for men with nonobstructive azoospermia

Study	Patients/cycles	Sperm retrieved per cycle	Clinical pregnancies per cycle
Devroey et al.[14]	15	12/15 (80%)	3/15 (20%)
Schlegel et al.[8]	16	10/16 (62%)	5/16 (31%)
Kahraman et al.[15]	29	14/29 (52%)	6/29 (21%)

Future directions

Further data will allow definition of how to more effectively extract sperm from men with nonobstructive azoospermia, and in vitro manipulation of retrieved sperm from men with obstructed systems may allow optimized results. The role of incompletely developed spermatozoa (that is, spermatids) for treatment of men without obstruction will also be clarified over time. Anecdotal pregnancies and deliveries in couples when the men have nonobstructive azoospermia have been reported using spermatids injected into the partner's oocytes after extraction of those spermatids from the ejaculate or testis. Early reports do not suggest that spermatids can frequently be extracted from the testis if TESE does not yield fully developed spermatozoa. 8 However, these areas of treatment are new, and spermatogonial transplants as well as the use of spermatids for fertilization have been reported in animal models. Since many men with nonobstructive azoospermia have marked genetic abnormalities, caution must be exercised before widespread application of these fertility treatments can be accepted as standard therapy.

Whether percutaneous and open sperm retrieval will have equal pregnancy results has yet to be determined. MESA procedures have a more reliable record of sperm retrieval and acquisition of sperm for cryopreservation. Slightly lower, but adequate sperm retrieval and good pregnancy results are achieved with percutaneous techniques with less morbidity. Most important, centers should not limit couples' options for treatment based on their own technical limitations, but always provide the option of simultaneous ICSI with sperm retrieval and the opportunity for microsurgical retrieval to limit the number of sperm retrieval procedures a man must endure.

Conclusions

Sperm retrieval from men with azoospermia is now possible with acceptable to excellent pregnancy rates when applied with intracytoplasmic sperm injection (ICSI). The ability to use cryopreserved epididymal and often testicular spermatozoa should continue to limit the number of sperm retrieval procedures necessary to achieve fertility for a couple. These advancements, both in sperm retrieval and assisted reproduction, provide the potential of fertility treatment where the only management options were donor insemination or adoption only several years ago. Specific genetic abnormalities are associated with azoospermia in men and careful evaluation of the cause of azoospermia is indicated for all men. Multiple TESE procedures may cause both transient and occasional permanent alterations in testicular function including testicular atrophy. Therefore, sperm retrieval should preferably be performed by physicians experienced in testicular anatomy and physiology.

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