

Chapter 11

Sperm Retrieval Techniques

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Introduction

The collection of sperm from the male genital tract was first described in 1985 [1]. But the procedures of sperm retrieval become an integral part of the management of azoospermia only after the report of a successful pregnancy by using testicular sperm extraction followed by intracytoplasmic sperm injection (ICSI) in 1993 [2]. While testicular sperm are retrieved from men with nonobstructive azoospermia (NOA), sperm may be retrieved from either the epididymis or testis in men with obstructive azoospermia (OA).

Cochrane meta-analysis has determined that there is insufficient data from trials to recommend any particular surgical sperm retrieval technique for either OA or NOA [3]. The complex interplay between male and female factors, and sperm retrieval and artificial reproductive technology (ART) means the management of infertile couples should be individualized.

In this chapter, we describe the preoperative preparation and postoperative care for patients undergoing sperm retrieval procedures. The principles of selection among different sperm retrieval techniques for patients with OA and NOA are illustrated by 2 clinical scenarios.

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Preoperative Preparation

Patients are instructed to withhold anticoagulants and antiplatelet medications for a week before the procedure. Abstinence from ejaculation for 2–3 days prior to the procedure is advised. Blood tests including screening of infectious diseases are performed. Laboratory tests for infectious disease status may consist of hepatitis, syphilis, and human immunodeficiency virus (HIV) and may vary between centers. A quarantine cryopreservation tank may be required in case of positive test results.

The vast majority of sperm retrieval procedures are performed on an outpatient basis. Shaving or clipping of the surgical site can be performed 1 day before or on the day of surgery [4]. Intravenous antibiotic with coverage of Gram-positive organisms is administered at least 30 min before skin incision except for percutaneous procedures. A bench microscope with appropriate containers and transport media should be available in the operating room for intraoperative examination of specimens. An experienced embryologist should be present, if necessary.

General anesthesia is generally preferred if the procedure involves the use of an operative microscope. Local anesthesia may be employed for percutaneous procedures, particularly in the outpatient setting. However, many patients reported significant discomfort and anxiety during percutaneous sperm retrieval procedures with spermatic cord block alone [5]. The co-administration of intravenous sedation would offer patients the additional benefits of an anxiolytic and possibly amnesia. Patients have reported greater satisfaction with the addition of intravenous sedation especially for bilateral or longer procedures [6].

Case Scenarios

Case 1

A 50-year-old gentleman, who had a vasectomy 15 years ago, wishes to have another child. His wife is 38 years old with a normal evaluation. They have 1 child together but also a history of 3 miscarriages. Testes measure 20 cc in volume bilaterally. Both epididymides are mildly prominent. The couple decides for sperm retrieval and ART instead of reconstructive surgery.

Diagnostic testing for post-vasectomy patients before sperm retrieval is generally not necessary. Hormonal evaluation with serum follicle-stimulating hormone (FSH) and testosterone is performed if there is clinical suspicion of impaired spermatogenesis.

Options of Sperm Retrieval Procedures

Sperm may be retrieved from epididymis or testis in azoospermic men after vasectomy. Epididymal sperm retrieval is the most commonly performed procedure in this situation. The procedure can be performed by percutaneous or open approaches. Percutaneous epididymal sperm aspiration (PESA) can be performed under local anesthesia, and an operative microscope is not required. It may be performed in an office setting. Patients recover quickly after the procedure with low complication rates. A needle is advanced percutaneously into the epididymal tubules. The needle is advanced in and out gently with negative suction force applied via a 30 cc or 60 cc syringe. Epididymal fluid is aspirated. Around 0.1 mL of fluid is usually obtained per aspirate [7]. The procedure is repeated at different sites from cauda to caput epididymis until an adequate number of motile sperm are retrieved. Since PESA is a blind procedure, multiple attempts may be required to obtain good quality sperm. Aspirates from cauda are often rich in senescent spermatozoa, debris, and macrophages. The phenomenon of better quality sperm at the proximal reproductive tract in men with chronic obstruction has been termed “inverted motility” [8]. It may be rational to start the procedure from corpus epididymis toward caput since vasectomy was performed 15 years ago. The concern about the detrimental effect of PESA on subsequent reconstructive microsurgery is not valid [9].

Epididymal sperm can also be acquired by an open approach with microscopic epididymal sperm aspiration (MESA). MESA involves incision of the epididymal tunica and inspection of the epididymal tubules. Dilated epididymal tubules with clear contents are punctured or incised, and the fluid is collected. A single MESA procedure usually enables retrieval of a great number of motile sperm [10], which are usually sufficient for cryopreservation for multiple subsequent in vitro fertilization (IVF) cycles [11]. The open procedure allows better hemostasis and decreased risk of hematoma formation compared to PESA [12]. Also, the contamination of the sample by red blood cells is minimized. The risk of scarring and epididymal obstruction after MESA is likely lower compared to PESA due to targeted aspiration of individual tubules under direct microscopic vision, and incised tubules can be repaired. MESA has been modified to combine advantages of percutaneous technique with precision of microsurgical procedure. The epididymis is brought anteriorly and examined via a 1–2-cm scrotal incision during the procedure of “mini-MESA” [13].

Testicular sperm can be retrieved by testicular sperm aspiration (TESA) percutaneously from men with OA. The testicular parenchyma is aspirated by fine needle, large-diameter needles, or tissue-cutting biopsy needles. Location of sperm aspiration matters little in terms of successful sperm retrieval. Sperm in obstructed testes is found throughout the parenchyma [14]. An entry point starting at the superior testicular pole and passing inferiorly and obliquely may carry less risk of vascular injury. Conversely, testicular sperm extraction (TESE) is rarely employed in men with OA. The pros and cons of various sperm harvesting techniques for OA is summarized in Table 11.1.

Table 11.1 Advantages and disadvantages of sperm retrieval techniques for obstructive azoospermia

	Advantages	Disadvantages
PESA	<ul style="list-style-type: none"> • Fast • Low cost • Possibly office/outpatient procedure • Minimal recovery and morbidity • Repeatable • No microsurgical skill and instruments required 	<ul style="list-style-type: none"> • Few sperm retrieved • May not retrieve adequate sperm for cryopreservation • May cause epididymal obstruction at puncture sites • Risk of hematoma formation
MESA	<ul style="list-style-type: none"> • Ample sperm retrieved • Excellent chance of sperm cryopreservation • Decreased risk of hematoma 	<ul style="list-style-type: none"> • Increased cost and anesthetic/operating time • Microsurgical skill and instruments required • Surgical exploration required with longer postoperative recovery
TESA	<ul style="list-style-type: none"> • Fast • Low cost • Possibly office/outpatient procedure • Minimal recovery and morbidity • Repeatable • No microsurgical skill and instruments required 	<ul style="list-style-type: none"> • May not retrieve adequate sperm for cryopreservation • Risk of hematoma formation • Risk of testicular atrophy
TESE	<ul style="list-style-type: none"> • Fast • Repeatable • No microsurgical skill and instruments required 	<ul style="list-style-type: none"> • Increased cost and operating time • Surgical exploration required with longer postoperative recovery • Risk of testicular atrophy

PESA percutaneous epididymal sperm aspiration; *MESA* microsurgical epididymal sperm aspiration; *TESA* percutaneous testicular sperm aspiration; *TESE* conventional testicular sperm extraction

Selection and Results of Sperm Retrieval Technique in Post-vasectomy Patients

The sperm retrieval rate (SRR) in patients with OA is high and ranges from 90 to 100% [15]. Successful PESA has been reported in 78.0% of the cases, and subsequent percutaneous testicular retrievals are successful in the vast majority of failed epididymal sperm retrievals. The cumulative success rate of percutaneous approaches in OA patients reaches 97.3% irrespective of the cause of obstruction [16]. A low SRR of around 20% has been reported when an epididymal cyst is present, which is a common finding after vasectomy [17, 18]. In this patient population, however, subsequent sperm retrieval by TESA or TESE still carries a high success rate.

The history of multiple miscarriages without an identifiable female factor in our clinical scenario also needs to be investigated and may have implication on the choice of sperm retrieval technique. The impact of paternal factors on reproductive

outcomes is increasingly being recognized. Sperm deoxyribonucleic acid (DNA) fragmentation has been widely studied in recent years and has been increasingly associated with recurrent pregnancy loss particularly in the setting of ART [19]. The association between aging and loss of DNA integrity [20] is particularly worrisome in our patient. Sperm DNA fragmentation (SDF) testing may be diagnostic in identifying the etiology of recurrent miscarriage, especially after ART failure. Testing may also provide prognostic information on ART outcomes for the couple. There is evidence to show that high SDF is associated with increased risk of pregnancy loss and decreased live birth [21]. Treatment strategies, including oral antioxidants and sperm selection, can be considered in case of elevated SDF levels. Testicular sperm retrieval with TESA or TESE may be preferable in patients with high SDF since the incidence of DNA fragmentation is markedly lower in testicular sperm [22, 23].

It is rational to start retrieval of epididymal and/or testicular sperm percutaneously (i.e., PESA ± TESA) in our patient with expected high SRR approaching 90–100% [24]. Percutaneous sperm retrieval provides the advantages of minimal invasiveness with low complication rate. The procedure can be performed in the office setting under local anesthesia without the use of operative microscope and microsurgical technique. PESA should start from corpus epididymis toward caput in view of the phenomenon of inverted motility. MESA should be considered if our patient desires a single sperm retrieval procedure and cryopreservation for multiple subsequent ART cycles [11, 25]. Retrieval of testicular sperm should be considered in the presence of epididymal cyst or high SDF.

Artificial Reproductive Technology Outcomes in Men with Obstructive Azoospermia

Pregnancy success rates utilizing epididymal sperm from patients with OA in intrauterine insemination (IUI) [26] and in vitro fertilization [1] have been reported. Good oocyte fertilization and pregnancy rates in ICSI have been achieved with epididymal sperm. Fertilization, clinical pregnancy, and live birth rates of 60, 50, and 35%, respectively, can be achieved [27]. The use of testicular sperm from men with OA in ICSI is also associated with similar high pregnancy rates [28]. The source of sperm and retrieval modality does not affect outcome of ART in our patient [29, 30]. ICSI outcomes using fresh or frozen-thawed sperm retrieved from men with OA are also comparable [20, 31].

Sperm quality in OA patients is generally high. If sperm quality or quantity from the epididymis is poor, consideration should be given to TESE. Cryopreservation and use of frozen-thawed sperm in ART will not compromise the reproductive outcomes in our patient.

Case 2

A 30-year-old gentleman has infertility and azoospermia on semen analysis. His 28-year-old wife has a normal evaluation. Testicular volume is 8 cc bilaterally with palpable vasa deferentia on physical examination of the patient. A grade 3 left varicocele was revealed on physical examination. Serum FSH and testosterone levels are 30 IU/L and 200 nmol/L, respectively. Testicular sperm retrieval has been attempted previously with no sperm retrieved, and there is no further detail available.

While retrieval of good quality sperm from men with OA is very likely, sperm retrieval success rates in men with testicular failure and NOA is much lower. Donor sperm insemination and child adoption were the options left to men with NOA a few decades ago. The finding of heterogeneous “patchy” spermatogenesis within the testes of approximately one-third of men with NOA on a single diagnostic biopsy provides the rationale in the management of NOA by sperm retrieval [32]. Despite the severely impaired spermatogenesis with inadequate sperm production to reach the ejaculate, sperm can be demonstrated within the testes in at least 60% of men with NOA in a more recent study [33]. Testicular sperm retrieval combined with ICSI in our patient offers the chance for the patient to father his own biologic children.

Preoperative Investigations and Optimization

Meticulous microscopic examination of the pellet is necessary to determine whether a semen sample is truly azoospermic. It is shown that sperm are identified in up to 35% of men who are thought to have NOA during an extended examination of a centrifuged specimen [34]. The definitive diagnosis of NOA relies on histological confirmation. However, a clinical diagnosis based on history, small testicular volume, and flat epididymides on physical examination, elevated serum FSH levels, and azoospermia on semen analysis can be made in many cases.

Diagnostic testicular biopsy remains the gold standard in differentiation between OA and NOA. However, the small samples obtained from diagnostic biopsy are unlikely to be representative since both testicular histology and sperm production are heterogeneous within the seminiferous tubules. Currently, many centers perform testicular biopsy for histology at the time of sperm acquisition. A separate procedure of testicular biopsy is not regarded as mandatory before sperm retrieval procedures by many male fertility specialists.

Karyotyping and Y-chromosome microdeletion (YCMD) testing typically identify the etiology of impaired spermatogenesis in 15–20% of NOA patients, and up to 17% of TESE candidates are found to have abnormal genetic evaluation [35]. YCMDs are more commonly detected in patients with lower sperm production. Ten percent of azoospermic men are noted to have YCMD, while no microdeletion is

detected in men with sperm counts more than 5×10^6 [36]. It is now possible for the transmission of defective genetic material to offspring with advent of ICSI and sperm retrieval. It is therefore advisable to have genetic evaluation before sperm retrieval. Results of genetic tests have been shown to alter the choice of treatment in 21% of infertile couples [35]. Donor sperm, adoption, and embryo biopsy are some options elected by patients after genetic counseling. Apart from genetic evaluation, elevated serum FSH level is one of the clinical features of men with NOA. The prognostic value of hormonal and genetic evaluation on sperm retrieval will be discussed later in this chapter. Imaging modalities are generally not indicated for the management of NOA patients unless there are abnormalities on physical examination.

Spermatogenesis should be optimized for at least 3 months prior to sperm retrieval. Any reversible causes should be corrected including avoidance to gonadal toxins. The role of varicocelectomy in our patient with NOA is not well defined. Most patients have no return of sperm to the ejaculate and require sperm retrieval despite repair of varicocele [37]. Varicocelectomy does not influence subsequent SRR in men with NOA and clinical varicoceles. The beneficial effect of varicocelectomy may take 6 months or longer to appear and, therefore, may not be a sensible choice for our patient. Hormonal disturbances, including compromised serum testosterone and increased estradiol levels, are common among men with NOA [38]. Testosterone-to-estradiol ratio (TE ratio) is commonly used clinically as an expression of the overall androgen and estrogen balance. The mean TE ratio in fertile controls is significantly higher compared to men with severe infertility [38]. Increased aromatase activity of the testes may contribute to the phenomenon [39]. By directly limiting estrogen feedback to the pituitary gland, aromatase inhibitors increase production of FSH and luteinizing hormone (LH). The correction of the endocrinopathy may enhance endogenous intratesticular testosterone levels and thus spermatogenesis. Both steroidal (testolactone) and nonsteroidal (anastrozole) aromatase inhibitors raise serum testosterone levels and correct TE ratios effectively [40]. A TE ratio of less than 10 is proposed as the cutoff to initiate treatment. Although significant improvements in the hormonal profile and semen parameters have been demonstrated in oligozoospermic men treated with testolactone, there are no studies that have demonstrated a return of sperm to the ejaculate in azoospermic men with treatment [38]. The use of aromatase inhibitors in men with NOA remains off-label. The correlation between hormone manipulation and fertility benefits remains to be defined by randomized controlled studies.

In summary, karyotyping and YCMD testing should be performed in our patient before sperm retrieval procedures. The test results carry important prognostic value. Varicocelectomy as an adjunct before sperm extraction has no evidence to improve SRR. Testing of estradiol level may be considered, and treatment initiated with TE ratio less than 10. However, the current evidence of correction of endocrinopathy with aromatase inhibitors in our patient with NOA is weak.

Procedures in Men with Previous Failed Sperm Retrieval

Failure of previous sperm retrieval does not deter further attempts in our patient. The characteristic of patchy foci of sperm production in men with NOA renders a single biopsy inadequate for identification of sperm most of the time. Multiple biopsies are essential for the successful sperm retrieval in men with NOA. Only 23% of men have sperm identified on the first biopsy, and up to 14 biopsies may be required to locate sperm in a single procedure of open testicular biopsy from 1 or both testicles [41]. Repeating testicular biopsy or testicular sperm aspiration (TESA) may be a less favorable option in view of the low SRR. Microdissection testicular sperm extraction (mTESE) after previous failed TESA or TESE procedures is more commonly practiced and studied. Patients who had 1 or 2 prior biopsies per testis have an SRR of 50% by mTESE. SRR decreases to 22% if 3 or more previous biopsies were performed per testis. This is compared to 52% of SRR with mTESE in patients who have no prior testicular surgery [42]. The minimal impact on subsequent SRR by 1 or 2 prior testicular biopsies strongly suggests that random testicular biopsies commonly miss areas of sperm production. The chance of sperm identification on repeated mTESE is 33% even when no sperm is found on the first mTESE [43]. Data show that mTESE achieves reasonable SRR after failed testicular biopsy, TESA, TESE, and mTESE in the hands of an experienced infertility surgeon. Repeated mTESE appears a viable option for our patient.

A 6-month interval between sperm retrieval procedures is recommended to our patient. This recommendation is based on the concept that spermatogenesis can be adversely affected by postoperative changes and sperm production may take 3 months to be fully restored. Although clinical data on the effect of the time interval between sperm retrieval procedures and SRR are lacking, the suggestion of a 6-month interval is supported by circumstantial evidence. It is found that 82% of abnormal sonographic findings of the testes at 3 months after TESE procedures resolve by 6 months [44]. The incidence of ultrasound findings suggestive of hematoma decreases from 5 to 7.5% and 12 to 2.5% at 1 and 6 months after conventional TESE and mTESE, respectively, suggesting that at least 6 months is needed for most of the testes to fully recover after sperm retrieval procedures [45]. However, the varying degree of testicular damage caused by different sperm retrieval procedures indicates that the optimal timing to repeat sperm retrieval procedures should be individualized. Serial ultrasound imaging of the testes may be helpful in defining the optimal time interval. While the majority of ultrasound abnormalities resolve by 6 months, endocrine function and serum testosterone level may take up to 18 months to recover [46]. The question remains unanswered, and the optimal time interval between sperm retrieval procedures is yet to be defined by further research.

Sperm Retrieval Procedures for Men with Nonobstructive Azoospermia

The testis is the only sperm source for our patient. There are several options available for testicular sperm retrieval: (1) TESA, (2) conventional TESE, (3) mTESE, and (4) testicular mapping. TESA attempts to retrieve sperm by percutaneous technique. The procedure can be performed under cord block and local anesthesia when a fine needle is used. The low SRR renders percutaneous procedures uncommon [47, 48]. TESA is not recommended as the primary procedure of sperm retrieval for men with NOA except when used in conjunction with testicular mapping. It has been shown that percutaneous procedures are less effective than open testicular biopsy in obtaining sperm [33, 49].

TESE and mTESE are open testicular biopsy techniques and are more commonly performed in men with NOA. Multiple biopsies are usually employed to locate sperm during conventional TESE [41]. Conventional multiple biopsy TESE achieves up to 50% SRR [50]. However, it carries the risk of damage to the testicular blood supply. Complete testicular devascularization has been reported after multiple biopsies.

Since the introduction of mTESE in 1999 [33], the procedure has gained popularity due to several advantages over conventional TESE. The use of a microscope allows identification of subtunical blood vessels and decreases the risk of damage to the testicular blood supply [51]. A higher SRR of 45–65% is associated with mTESE compared to 30–45% with conventional TESE [33, 47, 51]. Moreover, mTESE is more effective in recovering sperm from men with testicular volume of less than 10 mL [52]. Larger quantity of sperm is obtained during mTESE with less testicular tissue removed. An average of 160,000 spermatozoa are obtained in samples that weigh 9.4 mg during mTESE, compared to 64,000 spermatozoa yielded by 720 mg of testicular tissue from conventional TESE [33]. However, it was concluded in a systematic review that mTESE performs better than conventional TESE only in cases showing Sertoli-cell-only pattern on histology where tubules containing foci of active spermatogenesis can be identified by the microscopic appearance of larger and more opaque tubules [53].

mTESE also has the lowest complication rates compared to other sperm retrieval techniques [53]. mTESE results in less intratesticular reaction than conventional TESE despite the wide equatorial incision along the tunica albuginea and extensive dissection. The achievement of complete hemostasis during mTESE results in less acute and chronic sonographic changes on scrotal ultrasound. Less postoperative pain after mTESE has been reported due to less retraction of tunica albuginea and compression of testicular parenchyma [54].

Despite the advancement in sperm retrieval techniques, lasting effects on testicular function after testicular sperm extraction should not be overlooked. Serum testosterone levels drop by 20% of preoperative levels at 3–6 months after sperm retrieval procedures and are not completely recovered at 18 months postoperatively [46]. It also has been reported that mTESE leads to reduction in serum testosterone

levels and increase in FSH and LH levels [55]. Histologic studies of the testes after sperm extraction procedures reveal a 7 and 5% decrease in seminiferous tubule volume and germ cell density, respectively [56].

Another option for obtaining sperm from our patient with NOA is testicular mapping. It consists of systematic fine needle aspiration (FNA) following a 22-site template of bilateral testes. Further management is stratified by the test results. Patients who have no sperm identified are offered the options of adoption and donor insemination, and attempt to use a sperm retrieval procedure is generally not recommended in expert centers. On the other hand, a directed sperm retrieval procedure will be offered in the presence of sperm. The location and quantity of sperm identified on mapping guide the subsequent sperm retrieval procedures. Testicular mapping is an outpatient procedure performed under local anesthesia. The procedure is well tolerated, and patients usually resume normal activity within a day [57]. An early study has demonstrated the potential use of FNA to identify sperm in men with NOA with 2–3 samples from each testis [58]. The role of FNA is further supported by a report of 60% SRR in men with NOA with up to 15 samples from each testes, but the quantity is insufficient to inject all a partner's ova in most cases [59]. Therefore, testicular mapping/FNA as the sole sperm retrieval procedure is not recommended. The optimal number of sites of diagnostic aspiration remains unclear. Despite the advantage in avoiding or minimizing the invasiveness of sperm retrieval procedures, the wide application of testicular mapping is hindered by the significant cytologic experience required in identifying sperm in a smear of aspirated seminiferous tubules.

Subsequent sperm retrieval is executed from the least to most technically demanding procedures in the sequence of TESA, conventional TESE, and mTESE based on the map. It has been demonstrated that sufficient sperm for injection of all available oocytes can be retrieved in 95% of cases [60]. Bilateral procedure was only required in 22% of patients. Complex sperm retrieval with mTESE was performed in 23% of men, while the majority had sperm acquired by TESA and TESE [60]. It is of note that the high SRR was reported from patients with positive FNA results to begin with. Currently, there is no head-to-head studies comparing the different strategies of mTESE and testicular mapping \pm sperm retrieval. The advantages and disadvantages of various sperm retrieval techniques in men with NOA are presented in Table 11.2.

The importance of intraoperative specimen handling in increasing sperm yield has been addressed. The mechanical disruption of individual tubules by aggressive mincing in the medium and repeated passage of testicular suspension via angio-catheter increases sperm yield by up to 300-fold [61]. The procedure of sperm retrieval can be terminated once sufficient sperm are identified in the operating theater by surgeon or embryologist under microscope. The increased efficacy in sperm identification prevents unnecessary damage to the already compromised testis of our patient.

In summary, repeating sperm retrieval at least 6 months after the previous attempt is a rational approach for our patient. mTESE seems the preferred technique

Table 11.2 Advantages and disadvantages of sperm retrieval techniques for nonobstructive azoospermia

	Advantages	Disadvantages	Sperm retrieval rates (%)
TESA	<ul style="list-style-type: none"> • Fast • Low cost • Possibly office/outpatient procedure • Minimal recovery and morbidity • No microsurgical skill and instruments required 	<ul style="list-style-type: none"> • May not retrieve adequate sperm for injection of all retrieved oocytes • Risk of hematoma formation • Risk of testicular atrophy 	5–10
TESE	<ul style="list-style-type: none"> • No microsurgical skill and instruments required 	<ul style="list-style-type: none"> • Surgical exploration required with longer postoperative recovery • Risk of testicular atrophy 	30–45
mTESE	<ul style="list-style-type: none"> • Thorough examination of testicular parenchyma • Reduced risk of damage to testicular blood supply • Less testicular tissue removed • Less adverse effect on testicular function 	<ul style="list-style-type: none"> • Increased cost and operating time • Surgical exploration required with longer postoperative recovery • Microsurgical skill and instruments required 	45–65
Testicular mapping (\pm sperm retrieval)	<ul style="list-style-type: none"> • Possibly office/outpatient procedure • Minimal recovery and morbidity • No microsurgical skill and instruments required • Avoid morbidities associated with sperm retrieval procedures for patients with no sperm identified on testicular mapping • Potentially reduce the invasiveness of the subsequent sperm retrieval procedure 	<ul style="list-style-type: none"> • Significant cytologic experience required • Some patients are subjected to 2 procedures • Possible false negative despite extensive systematic fine needle aspirations 	95 ^a

TESA percutaneous testicular sperm aspiration; *TESE* conventional testicular sperm extraction; *mTESE* microdissection testicular sperm extraction

^aPatients with sperm identified on testicular mapping

and has been more widely studied as the procedure after failed TESA/conventional TESE/mTESE attempts and showed promising results. The technique has also been suggested to be particularly useful for our patient with small testicular volume. mTESE may have less of a detrimental impact on testicular function. There is more rapid recovery of hormonal profile and resolution of sonographic abnormalities after mTESE. Meticulous specimen handling intraoperatively is of paramount importance in maximizing the sperm yield. The alternative of testicular mapping \pm sperm retrieval can be considered if significant cytologic expertise is available.

Prognostic Factors

The success of surgical sperm retrieval in men with NOA is variable. Early studies in identifying prognostic factors for successful sperm retrieval have been disappointing. Clinical features, including testicular volume, history of ejaculated sperm, serum FSH or inhibin levels, etiology of NOA, and biopsy histology, do not predict success of sperm retrieval procedures [62, 63]. More recent data suggest that YCMD and histopathologic diagnosis are the most promising predictive factors. The presence of AZFc in azoospermic men is considered a favorable factor associated with a SRR of 71.4% compared to 48.8% retrieval rate in patients with idiopathic azoospermia [64]. The clinical pregnancy rates per IVF cycle involving sperm retrieval from men with AZFc microdeletion are comparable to that of unaffected individuals [65]. On the other hand, sperm retrieval is universally unsuccessful in all patients with complete AZFa or AZFb deletions [66].

Histopathologic diagnosis may be helpful in predicting treatment success in case prior diagnostic biopsy has been performed. The most advanced stage on biopsy, but not the predominant stage, is considered as the predictive factor [63]. It has been shown that sperm are identified in 81, 44, and 41% of patients with hypospermatogenesis, maturation arrest, and Sertoli-cell-only, respectively, by using mTESE [46]. Correlations between SRR and histopathologic diagnosis are also demonstrated with standard open testicular biopsy [67] and testicular FNA techniques [59]. A study suggested that the presence of Sertoli-cell-only on biopsy as the most advanced pattern in men with at least 1 prior failed sperm retrieval is associated with lower SRR [42]. Other factors have been suggested to have prognostic value on SRR as well. The role of serum FSH level as a predictive factor of successful sperm retrieval is less well defined. One study has demonstrated that a cutoff level of serum FSH > 20 IU/L predicts successful sperm retrieval with open biopsy methods [68]. Conversely, other studies have demonstrated that serum FSH levels are less relevant for predicting success of mTESE. Patients who have serum FSH of 15–30 IU/L, 30–45 IU/L, or greater than 45 IU/L all have similar SRRs [69].

The negative effect of prior biopsies on conventional TESE is suggested by 56% SRR in men who underwent no prior biopsy compared to 23% SRR for those who had 3–4 biopsies per testis [41]. The phenomenon may be explained by scarring and parenchymal fibrosis as a result of devascularization by multiple biopsies. Prior success in sperm retrieval predicts good SRR on repeat procedures. The SRR reaches 96% on repeated mTESE following prior successful retrieval. On the other hand, the SRR drops to 33% if sperm is not found on previous mTESE [43].

It also has been proposed that the response to aromatase inhibitor in men with Klinefelter syndrome predicts the results of sperm retrieval [70]. Whether the result can be extrapolated to other non-Klinefelter syndrome men with NOA and low TE ratio is unknown. Table 11.3 is a summary list of the possible prognostic factors for sperm retrieval in men with NOA.

Therefore, obtaining the details of the previous sperm retrieval procedure is of paramount importance in addition to YCMD test results in our patient. Although a

Table 11.3 Prognostic factors for successful sperm retrieval in men with nonobstructive azoospermia

Y-chromosome microdeletion	Complete deletion of AZFa or AZFb are extremely poor prognostic factors Presence of AZFc is associated with sperm retrieval rate of approximately 70%
Histopathologic diagnosis	Sertoli-cell-only pattern is generally associated with lower sperm retrieval rate compared to hypospermatogenesis and maturation arrest
Serum follicular-stimulating hormone (FSH)	FSH > 20 IU/L signifies remote chance of sperm retrieval with conventional multifocal testicular sperm extraction (TESE) Serum FSH does not predict sperm retrieval rate during microdissection testicular sperm extraction (mTESE)
Previous testicular biopsies	More than 2 prior biopsies per testis is associated with reduced chance of sperm retrieval
Results of prior sperm retrieval	Prior success in mTESE is associated with 96% sperm retrieval rate in repeated procedure Repeating mTESE in patients with previous failed attempt is associated with 33% sperm retrieval rate
Response to aromatase inhibitors	The role of aromatase inhibitor in non-Klinefelter syndrome men is unclear

prior failure of sperm retrieval predicts lower success on subsequent procedures, other information such as the surgical technique of previous attempts and histopathologic diagnosis also carries prognostic value.

Fresh Versus Cryopreserved Retrieved Testicular Sperm

There has been considerable debate between using fresh versus frozen testicular sperm for ART in men with NOA. A meta-analysis concludes that fertilization rates, clinical pregnancy rates, and ongoing clinical pregnancy rates do not differ between groups using fresh or cryopreserved testicular sperm from men with NOA [15]. Some authors also suggest cryopreservation of retrieved testicular sperm followed by ICSI later in order to avoid unnecessary ovarian stimulation of the female partner [71]. But there is a concern of using cryopreserved testicular sperm for ICSI based on the finding that only 33% of testicular samples from men with NOA show documentable viability after freeze-thaw [72]. Currently, many fertility specialists prefer fresh to freeze-thawed testicular sperm. Coordinated IVF cycles and sperm extraction procedures are required in order to use fresh testicular sperm. Fresh testicular sperm has a high viability rate approaching 90% despite its low motility. Injection of nonmotile fresh testicular sperm during ICSI yields a high fertilization rate [73]. It is now recognized that the motility of retrieved testicular sperm remains stable or increases with incubation *in vitro* for 24–48 h [74]. This has simplified the timing of procedures on infertile couples, and testicular sperm can be retrieved 1–2 days before ICSI.

Postoperative Care

Postoperative care differs between centers and surgeons. Ice packs may be applied intermittently to the scrotum for 24–48 h. Patients are strongly advised to wear briefs or a scrotal supporter until edema and pain subside. Scrotal swelling, wound ecchymosis, and discomfort usually subside in approximately 7 days. Normal daily activities can be resumed on the next day after percutaneous sperm retrieval and 3 days after open procedures. Men can begin showers after 24 h. Strenuous exercise should be avoided for 7–10 days. No sexual activity is recommended for 3–7 days. Antibiotic after the procedure is not necessary and not routinely prescribed [75], but some surgeons may prefer empirical oral antibiotic for 3–5 days. Pain medication is used as needed. Common prescription including narcotics or nonsteroidal anti-inflammatory medications is usually adequate for pain control.

Conclusion

The acquisition of satisfactory surgical sperm retrieval technique is essential for all male fertility specialists. Currently, there is insufficient evidence from randomized trials to recommend any particular procedure for both obstructive and nonobstructive azoospermia. A variety of procurement procedures are available, and the choice of techniques varies among centers. The formulation of a protocol for sperm retrieval at a particular center largely depends on the expertise and equipment available. The collaboration and discussion among male fertility specialists, ART specialists, and embryologists is essential. Choosing the right surgical approach can only be made with a thorough understanding of the pros and cons of each sperm extraction technique.

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