Sperm Retrieval Techniques

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INTRODUCTION

Within the last few decades there were 2 major achievements in the area of male infertility.[1–3] The first was the introduction of intracytoplasmic sperm injection (ICSI) for the treatment of male factor infertility due to severely abnormal semen quality.[1] The second was the extension of ICSI to azoospermic males and the demonstration that spermatozoa retrieved from either the epididymis or the testis were capable of normal fertilization and pregnancy.[2,3]

Azoospermia, defined as the complete absence of spermatozoa in the ejaculate after centrifugation, is found in 1–3% of the male population and in approximately 10% of the infertile males. Although azoospermia is associated with infertility, it does not necessarily imply sterility because many azoospermic men maintain sperm production at varying levels within the testes.[4] Several sperm retrieval methods have been developed to collect sperms from the epididymis or the testis of azoospermic men. Surgically retrieved-spermatozoa can be used to induce conception through assisted reproductive techniques (ART), i.e., in vitro fertilization (IVF) associated with ICSI.[1–7]

The aims of this chapter are 3-fold: (i) To review the methods for retrieval of epididymal and testicular spermatozoa, and their success rates in different clinical conditions; (ii) to provide a critical appraisal of the advantages and limitations of the current surgical methods to retrieve sperm for males with obstructive azoospermia (OA) and non-obstructive azoospermia (NOA); and (iii) to discuss the reproductive potential of the male gamete extracted from the epididymis or the testis, and used for assisted conception.

ETIOPATHOGENESIS OF AZOOSPERMIA

The choice of sperm retrieval technique and its success rates are dependent on the type of azoospermia being obstructive or non-obstructive. It is therefore important to determine a pre-operative diagnosis not only to define the best treatment strategy but also to allow proper counseling. Clinical history, physical examination, and laboratory tests for endocrine assessment (serum follicle-stimulating hormone [FSH] and testosterone levels) are useful tools for this purpose. Together, these factors provide about 90% prediction of the azoospermia type.[5] In OA spermatogenesis is intact but a mechanical blockage exists somewhere between the epididymis and the ejaculatory duct. Acquired OA include vasectomy, failure of vasectomy reversal, post-infectious diseases, surgical procedures in the scrotal, inguinal, pelvic or abdominal regions, and trauma.[4,6] Congenital causes of OA include cystic fibrosis, congenital absence of the vas deferens (CAVD), ejaculatory duct or prostatic cysts, and Young's syndrome.[4–7] Non-obstructive azoospermia comprises a spectrum of testicular histopathology patterns resulting from various causes that include

environmental toxins, medications, genetic and congenital abnormalities, varicocele, trauma, endocrine disorders, and idiopathic reasons.[4–7]

DIAGNOSIS

Men with OA usually have normal sized testes and hormone profile. Occasionally, the epididymis or the seminal vesicles may be enlarged or a cyst can be palpable on rectal examination. The presence of a low volume (<1.5 mL) acidic (pH < 7.0) azoospermic ejaculate, with absent or low fructose and epididymal thickening, associated to nonpalpable vasa deferentia is pathognomonic of OA[4,6] Approximately two-thirds of men with OA and CAVD have mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Failure to identify a CFTR abnormality in a man with CAVD does not rule out the presence of a mutation, since some are undetectable by routine testing methods. The female partner should be offered cystic fibrosis (CF) testing before proceeding with treatments that utilize the sperm because of the high risk of the female being a CF carrier. If a CFTR gene mutation is identified (~4% of female partners are carriers), counseling is recommended before proceeding with sperm retrieval and ICSI due to the risk of the transmission of cystic fibrosis to the offspring.[4,6–9] Azoospermic men with idiopathic obstruction and men with a clinical triad of chronic sinusitis, bronchiectasis, and OA (Young's syndrome) may be at higher risk for CF gene mutations as well. In such cases, testing for CF mutations and counseling is also advisable.[4,6]

Men with FSH levels, testicular size, and ejaculate volume within normal ranges may have either NOA or OA.[4,6–9] In such cases, a testicular biopsy may be required to provide a definitive diagnosis. Histopathological evaluation of testicular specimens indicates the presence of normal spermatogenesis in cases of OA while hypospermatogenesis or maturation arrest or Sertoli cell-only (SCO) are seen in men with NOA. A testicular biopsy may be dismissed in cases of elevated FSH and small testes because this association is indicative of NOA.[4,7] However, a biopsy may be considered to determine the likelihood of sperm retrieval in ICSI candidates with NOA. The presence of either spermatozoa on a wet prep or hypospermatogenesis on testicular histopathology is highly predictive of successful sperm retrieval in future retrieval attempts.[8,10] Conversely, the absence of sperm in a biopsy specimen does not absolutely exclude the chances of finding sperm elsewhere within the testis due to the heterogenic distribution of spermatogenesis in NOA men.[5,8,10]

Karyotyping and Y-chromosome microdeletion testing should be offered to men with NOA of unknown origin. Karyotypic abnormalities affect 10–15% of men with NOA, and the Klinefelter syndrome (KS) accounts for approximately two-thirds of the cases.[8,11] Y-chromosome infertility is seen in 7–15% of men presenting with NOA. Genetic testing may provide prognostic information for sperm retrieval.[5–8] Azoospermic patients with Y-chromosome microdeletions restricted to the azoospermia factor c (AZFc) region may harbor viable sperm within the testis. In contrast, the chances of finding sperm in men with complete azoospermia factor a (AZFa) or azoospermia factor b (AZFb) deletions is virtually zero.[12,13]

MANAGEMENT

Table 1 summarizes the commonly used methods to retrieve sperm and their indications.

Percutaneous Sperm Retrieval Methods

Craft and Shrivastav, in 1994, first described the use of the percutaneous approach to retrieve sperm from the epididymis.[14] Two years later, Lewin et al reported the use of testicular fine-needle aspiration to retrieve sperm from the testis.[15] Percutaneous retrievals are usually undertaken under local anesthesia only or in

Table 1.
Sperm Retrieval Techniques and Indications

Technique	Acronym	Indications
Percutaneous epididymal sperm aspiration	PESA	OA cases only
Microsurgical epididymal sperm aspiration	MESA	OA cases only
Testicular sperm aspiration	TESA;TEFNA[a]	Failed PESA in OA Epididymal agenesis in CAVD cases Favorable testicular histopathology[b] in NOA Previous successful TESA attempt in NOA
Testicular sperm extraction (single or multiple biopsies)	TESE	Failed PESA or TESA in OA NOA cases
Microsurgical testicular sperm extraction	Micro-TESE	NOA cases only

[a]TEFNA is a technical variation of TESA.

[b]Hypospermatogenesis.

PESA: percutaneous epididymal sperm aspiration, MESA: microsurgical epididymal sperm aspiration, TESA: testicular sperm aspiration, TESE: testicular sperm extraction, micro-TESE: microsurgical testicular sperm extraction, OA: obstructive azoospermia, NOA: non-obstructive azoospermia, TEFNA: testicular fine-needle aspiration.

association with intravenous (i.v.) sedation. Percutaneous sperm retrieval can be either diagnostic or therapeutic. In the former, it is used to confirm the presence of viable spermatozoa prior to ICSI. In the latter, it is carried out at the same day of oocyte retrieval or the day before.

Percutaneous Epididymal Sperm Aspiration

For percutaneous epididymal sperm aspiration (PESA), a fine-needle attached to a tuberculin syringe is inserted through the scrotal skin into the epididymis. Negative pressure is created by pulling the syringe plunger while the tip of the needle is gently moved in and out inside the epididymis until a clear fluid is seen coming into the syringe. The amount of fluid aspirated is often minimal (~0.1 mL), except in cases of CAVD in which 0.3–1.0 mL may be obtained. The aspirate is flushed into a tube containing warm sperm medium. The tube containing the epididymal aspirate is taken to the laboratory for immediate microscopic examination. Percutaneous epididymal sperm aspiration is repeated at a different site (from cauda to caput epididymis) until adequate number of motile sperm is retrieved. If PESA fails to retrieve motile sperm, testicular sperm retrieval can be attempted at the same operative setting.

Testicular Sperm Aspiration

Despite of minor technical variations, the common principle of all methods described for testicular sperm aspiration (TESA) involves the needle insertion through the scrotal skin into the testis. Then, testicular parenchyma is percutaneously aspirated using fine (e.g., 22 gauge) or large diameter (e.g., 18 gauge) needle. The needle is usually inserted at the anteromedial or anterolateral portion of the superior testicular pole, in an oblique angle towards the medial and lower poles. These areas are least likely to contain major branches of the testicular artery running superficially underneath the albuginea. Loupe-magnification may be used to avoid small vessels seen through the

skin. Negative pressure is created by pulling the syringe plunger while the tip of the needle is moved in and out the testis in an oblique plane to disrupt the seminiferous tubules and sample different areas. The specimen is flushed into a tube containing warm sperm medium, and is immediately transferred to the laboratory for microscopic examination. Testicular sperm aspiration or testicular sperm extraction (TESE) may be performed at the contralateral testis if insufficient or no sperms are obtained.

Microsurgical Sperm Retrieval Techniques

Microsurgical sperm retrieval can be performed under either local anesthesia in association with i.v. sedation or epidural anesthesia. Operating microscope and microsurgery technique are used throughout the procedures.

Microsurgical Epididymal Sperm Aspiration

Microsurgical epididymal sperm aspiration (MESA) was first described by Temple-Smith et al in 1985.[16] The surgical technique involves the exteriorization of the testis through a 2–3 cm transverse scrotal incision. The epididymal tunica is incised and an enlarged tubule is then dissected and opened with microsurgical scissors. Fluid exuding from the tubule is aspirated with the aid of a silicone tube or blunted needle attached to a tuberculin syringe. The aspirate is flushed into a tube containing warm sperm medium and is transferred to the laboratory for examination. Microsurgical epididymal sperm aspiration is repeated at a different site of the same epididymis (from cauda to caput) and/or at the contralateral one until adequate number of motile sperm is retrieved. If MESA fails to retrieve motile sperm, TESA or TESE can be performed at the same operative setting.

Microsurgical Testicular Sperm Extraction

Microsurgical-guided testicular sperm extraction (micro-TESE) was originally described by Schlegel in 1999.[17] Upon testis delivery, a single, large, mid-portion, incision is made in an avascular area of the tunica albuginea under 6–8× magnification and the testicular parenchyma is widely exposed. Dissection of the testicular parenchyma is undertaken at 16–25× magnification searching for enlarged islets of seminiferous tubules (more likely to contain germ cells and eventually normal sperm production). The superficial and deep testicular regions may be examined, if needed, and microsurgical-guided testicular biopsies are performed by carefully removing enlarged tubules using microsurgical forceps. If enlarged tubules are not seen, then any tubule different than the remaining ones in size is excised. If all tubules are identical in appearance, random microbiopsies are performed at each testicular pole. The excised testicular tissue specimens are placed into the outer-well Petri dish containing sperm media. Specimens are washed grossly to remove blood clots and are sent to the laboratory for processing and search for sperm. Albuginea and scrotal layers are closed using non-absorbable and absorbable sutures, respectively.

Conventional Testicular Sperm Extraction

Extraction of testicular parenchyma for sperm search and their use in association with ICSI was first described by Devroey and colleagues in 1995.[3] For conventional TESE, a standard open surgical biopsy technique is used to retrieve sperm without the aid of optical magnification. Testicular sperm extraction can be performed under either local anesthesia with/without i.v. sedation or epidural anesthesia, and it is often carried out using the 'window' technique. Briefly, a transverse 2-cm incision is made through the anterior scrotal skin, dartos, and tunica vaginalis. A small self-retaining eyelid retractor is placed to improve exposure of the tunica albuginea, since the testis is not exteriorized. The albuginea is incised for approximately 1 cm. Gentle pressure is made

onto the testis to extrude testicular parenchyma. A small fragment (approximately 5 × 5 mm) is excised with sharp scissors and placed promptly in sperm culture media. A single or multiple specimens can be extracted from the same incision. Alternatively, individual albuginea incisions can be made onto the upper, middle, and lower testicular poles to extract multiple biopsy specimens. Testicular specimens are sent to the laboratory for processing and immediate microscopic examination. Albuginea is closed using non-absorbable sutures.

PERSONAL CLINICAL EXPERIENCE

Out of 2,136 males seeking infertility evaluation at our tertiary center in Brazil from 2002 to 2009, 142 (6.6%) and 176 (8.2%) had OA and NOA, respectively, and underwent sperm retrieval either for diagnostic or therapeutic purposes.[6]

In our hands, PESA and TESA have been highly effective methods for retrieving sperm in the group of men with OA. Successful sperm retrieval (SRR) was achieved in over 85% of the cases using PESA, but more than one aspiration was required in approximately 40% of the cases.[6] In cases of failed PESA, TESA was found to be adequate to obtain sperm in practically all cases. Motile spermatozoa were obtained in approximately 73% of the cases after the first or second PESA aspirations, and TESA was carried out as a rescue procedure after failed PESA in about 14% of the individuals.[6]

We commence aspiration at the corpus epididymis, and proceed to the caput if necessary, since aspirates from the cauda are usually rich in poor quality senescent spermatozoa, debris, and macrophages. Epididymal sperm retrieval may fail in certain cases of epididymal fibrosis caused by multiple previous retrieval attempts or post-infection. In such cases, sperm retrieval can be attempted in the contralateral epididymis or in the testis.[6]

Our cumulative successful retrieval rate after percutaneous aspirations was 97.9%, in a recent series of 142 men with OA.[18] Rescue TESA yielded approximately 90% success rate in cases of failed PESA.[18] In our study, sperm retrieval success rates using percutaneous techniques were similar regardless of the cause of obstruction being vasectomy, congenital bilateral absence of the vas deferens (CBAVD), and post-infectious etiology categories.[18] Intracytoplasmic sperm injection provides fertilization rates of ~60–70% per injected oocyte when epididymal or testicular spermatozoa from men with OA are used.[18,19] In such cases, our clinical pregnancy and live birth rates are 45% and 35%, respectively.[18,19]

In the group of men with NOA, SRR rates are approximately 50% in most etiology-specific causes of NOA.[10] Testicular histopathology results are predictive of sperm collection using both TESA and micro-TESE. According to our data, overall SRR rates by TESA are low in cases of SCO (20%) and maturation arrest (33%). Conversely, SRR by TESA is approximately 100% in NOA men presenting with hypospermatogenesis on testicular histology, and 80% in those with a history of previous successful TESA attempt. Using micro-TESE, overall SRR rates are approximately 53%, but higher than TESA in cases of maturation arrest and SCO.[10] Intracytoplasmic sperm injection provides fertilization rates of ~50% per injected oocyte when testicular spermatozoa from men with NOA are used.[19] In such cases, our clinical pregnancy and live birth rates are 35% and 25%, respectively.[19]

Our approach is to perform TESA only in the favorable prognosis cases mentioned before. If TESA fails, however, we neither perform a second aspiration in the same testis, at the same operative time, nor convert it to an open procedure to avoid the risk of hematoma and testicular injury. Extensive bleeding is often seen during a rescue TESE after a failed TESA. Therefore, enlarged seminiferous tubules are difficult to identify even using the operating

microscope. In these occasions, we opt to perform TESA or TESE at the contralateral testis. For NOA patients without previous diagnostic testicular biopsy or TESA attempt, our choice is to perform sperm extraction using micro-TESE. Selection of spermatozoa from a smaller population of contaminating testicular cells allows more ease and greater speed for sperm pick-up and injection process, as well as alleviates contamination and blockage of the injection needle with cells and debris. It is far less technically demanding and labor-intensive to extract spermatozoa from small volume specimens than large pieces of testicular tissue that must be dissected, red blood cells lysed, and the rare spermatozoa searched for in a tedious fashion under an inverted microscope. Testicular sperm extraction sperm processing may be incredibly labor-intensive and the searching process may miss the rare spermatozoa within a sea of seminiferous tubules and other cells. Testicular sperm extraction/ micro-TESE may be scheduled either for the day of oocyte collection and ICSI or the day before. In a previous study, we observed that optimal fertilization by ICSI using surgically-retrieved sperm is obtained when the time frame from human chorionic gonadotropin (hCG) administration to microinjection does not exceed 44 hours.[20] Testicular tissue sperm processing, searching, and selection of viable spermatozoa for ICSI may take several hours in NOA cases. Our laboratory takes approximately 11.6 minutes to handle a single testicular spermatozoon from processing to microinjection in NOA, but only 5.5 minutes in OA. In other words, the average time required to perform ICSI in a standard NOA treatment cycle involving 8–12 metaphase-II oocytes is approximately 2 hours. For these reasons, we elect to perform micro-TESE the day before oocyte collection when a busy next day IVF laboratory workload is anticipated.

Our data show that the overall SRR, defined as successful surgical collection of spermatozoa, are significantly higher in group of OA (SSR = 97.9%; N = 139/142) compared to NOA (SSR = 61.9%; N = 109/176) ($P < 0.001$).[21] According to our results, the chances of retrieving spermatozoa (odds ratio = 43.0; 95% confidence interval 10.3–179.5) and of achieving a live birth by ICSI (OR = 1.86; 95% CI: 1.03-2.89) were markedly increased in couples whose male partner had obstructive rather than NOA.[21]

Table 2 highlights the advantages and disadvantages of most sperm retrieval techniques used.

The concept of cryopreservation may be used in association with sperm retrieval procedures. We routinely freeze excess motile epididymal spermatozoa, which are not needed for the current ICSI cycle. Most often, motile sperm will be available after thawing in such cases, and ICSI outcomes using motile fresh or frozen epididymal sperm seems not to differ. If only immotile spermatozoa are obtained, a method for selecting viable sperm for ICSI may be used. Our preferred method for selecting immotile viable sperm for ICSI is the sperm tail flexibility test,[22] since the hypo-osmotic swelling test is not appropriate for assessing sperm viability in cryopreserved specimens.[23] Cryopreservation of testicular sperm is also advisable, especially for men with NOA who often require multiple ICSI attempts to conceive but may not have an adequate number of sperm available for repeated retrieval attempts. However, post-thaw testicular sperm are often immotile or exhibit only a twitching motility, and ICSI results using immotile testicular sperm tend to be lower than fresh ones. Different strategies can be developed according to the results of each group. If freezing of surgically-retrieved specimens provides results similar to those with the use of fresh sperm, then the use of freezing specimens would be preferable. If not, fresh specimens are preferable. Currently, our cryopreservation technique for surgically retrieval sperm is the standard liquid nitrogen vapor method using TEST-yolk buffer and

Table 2
Advantages and Disadvantages of Sperm Retrieval Techniques

	Advantages	Disadvantages
PESA	Fast and low-cost Minimal morbidity, repeatable No microsurgical expertise required Few instruments and materials No surgical exploration	Few sperm retrieved Cryopreservation limited Fibrosis and obstruction at aspiration site Risk of hematoma/spermatocele
MESA	Large number of sperm retrieved Excellent chance of sperm cryopreservation Reduced risk of hematoma Reconstruction possible[a]	Surgical exploration required Increased cost and time-demanding Microsurgical instruments and expertise required Postoperative discomfort
TESA	Fast and low cost Repeatable No microsurgical expertise required Few instruments and materials No surgical exploration Minimal/mild postoperative discomfort	Relatively low success rate in NOA Few sperm retrieved in NOA Cryopreservation limited Risk of hematoma/testicular atrophy
TESE	No microsurgical expertise required Fast and repeatable	Relatively low success rate in NOA Relatively few sperm retrieved in NOA Risk of testicular atrophy (with multiple biopsies) Postoperative discomfort
Micro-TESE	Higher success rates in NOA[b] Larger number of sperm retrieved[b] Relatively higher chance of sperm cryopreservation[b] Low risk of complications	Surgical exploration required Increased cost and time-demanding Microsurgical instruments and expertise required Postoperative discomfort

[a]In cases of vasectomy.
[b]Compared to TESA and TESE in NOA.
PESA: percutaneous epididymal sperm aspiration, MESA: microsurgical epididymal sperm aspiration, TESA: testicular sperm aspiration, TESE: testicular sperm extraction, micro-TESE: microsurgical testicular sperm extraction, NOA: non-obstructive azoospermia.

glycerol as cryoprotectants.[24] Epididymal specimens are concentrated by washing before freezing, and testicular sperm are freed from the testicular parenchyma, i.e., testicular homogenates are frozen.

CONCLUSION

The goals of sperm retrieval are to obtain the best quality sperm possible in adequate numbers for immediate use and/or potential cryopreservation, while minimizing the damage to the reproductive tract. Sperm production is normal and gametes can be easily retrieved from the epididymis or testis in cases of OA. In OA, the choice of sperm retrieval by method and site of collection should be based upon preferences and expertise, since there is no evidence that either percutaneous or microsurgery from the testis or epididymis affects outcomes of sperm retrieval and assisted reproduction. Conversely, sperm production

can be either markedly impaired or absent in men with NOA. As such, open surgical testicular sperm retrieval with/without microscopic magnification is recommended for patients with NOA to optimize the chances of finding sperm. The sperm retrieval method has no impact on ART outcome for patients with NOA. However, the reproductive potential of azoospermic men who are candidates for sperm retrieval and ICSI is related to the type of azoospermia. The chances of retrieving spermatozoa and of achieving a live birth by ICSI are increased in couples whose male partner had OA rather than NOA. Children conceived using sperm retrieved from men with OA and NOA should be followed-up because it is still unclear if there is an increased risk of birth defects when ICSI is carried out with non-ejaculated sperm.

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