Chapter 5

Sperm retrieval techniques

Sandro C. Esteves and Ashok Agarwal

Introduction

Two major breakthroughs occurred in the area of male infertility only 2 to 3 years apart [1–3]. The first was the development 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 derived from either the epididymis or the testis were capable of normal fertilization and pregnancy [2, 3]. Azoospermia is defined as an absence of spermatozoa in the ejaculate after centrifugation. This condition, which is found in 1–3% of the male population and approximately 10% of infertile males, results in infertility but does not necessarily imply sterility [4]. In the case of azoospermia, two totally different clinical situations exist, i.e. obstructive and non-obstructive azoospermia. In obstructive azoospermia (OA), spermatogenesis is normal but a mechanical blockage exists in the genital tract, somewhere between the epididymis and the ejaculatory duct, or the epididymis and vas deferens are totally or partially absent. Causes of OA may be acquired or congenital. Acquired OA may be due to vasectomy, failure of vasectomy reversal, post-infectious diseases, surgical procedures in the scrotal, inguinal, pelvic, or abdominal regions, and trauma. Congenital causes of OA include cystic fibrosis, congenital absence of the vas deferens (CAVD), ejaculatory duct or prostatic cysts, and Young's syndrome [4]. Non-obstructive azoospermia (NOA) comprises a spectrum of testicular histopathology resulting from various causes that include environmental toxins, medications, genetic and congenital abnormalities, varicocele, trauma, endocrine disorders, and idiopathic. In both OA and NOA, pregnancy may be achieved through assisted reproductive techniques, i.e. in vitro fertilization associated with ICSI [4–5].

Several sperm retrieval methods have been developed to collect epididymal and testicular sperm for ICSI in azoospermic men. Either percutaneous (PESA) [6] or microsurgical epididymal sperm aspiration (MESA) [2] can be successfully used to retrieve sperm from the epididymis in men with obstructive azoospermia. Testicular sperm aspiration (TESA) can be used to retrieve sperm from the testes in men with OA who fail PESA as well as in those with NOA [7]. Testicular sperm extraction (TESE) using single or multiple open biopsies [8, 9] and more recently microsurgery (micro-TESE) are indicated for men with NOA [9–12]. Sperm can be easily obtained from infertile men with OA for ICSI whereas individuals exhibiting NOA have historically been the most difficult to treat. It is out of the scope of this chapter to provide a step-by-step laboratory description of the commonly used methods for PESA/TESA/TESE sperm processing and identification of viable immotile sperm for ICSI. However, as a general rule, processing of surgically retrieved spermatozoa should not only ease the selection of the best-quality spermatozoa for ICSI but also optimize the fertilizing ability of these often compromised specimens, particularly in the cases of NOA and after the freeze-thawing process.

This chapter describes surgical methods for retrieval of epididymal and testicular spermatozoa in men with obstructive or non-obstructive azoospermia. Sperm retrieval rates using different methods and in several clinical conditions are also presented, as well as clinical outcomes of ICSI using testicular and epididymal sperm.

Human Assisted Reproductive Technology: Future Trends in Laboratory and Clinical Practice, ed. David K. Gardner, Botros R. M. B. Rizk, and Tommaso Falcone. Published by Cambridge University Press.

Step-by-step description of surgical techniques

Percutaneous sperm retrieval techniques

Anesthesia

Percutaneous sperm retrieval is carried out under local anesthesia only or in association with intravenous sedation. In both cases, a 10 ml solution of 2% lidocaine is injected around the spermatic cord near the external inguinal ring. In cases where intravenous anesthesia is used, local injection of the anesthetic is performed after patient unconsciousness is achieved.

Percutaneous epididymal sperm aspiration (PESA)

Indications

PESA is indicated in obstructive azoospermia cases only.

Technique

- After anesthetic blockade of the spermatic cord, epididymis is stabilized between the index finger, thumb and forefinger while the testis is held with the palm of the hand.
- A 13-gauge needle attached to a 1 ml tuberculin syringe is inserted into the epididymis through the scrotal skin. Loupe-magnification is used to avoid injuring small vessels seen through the skin (see Figure 5.1A).
- Negative pressure is created and the tip of the needle is gently moved in and out within the epididymis until fluid enters the syringe. The amount of epididymal fluid obtained during aspiration is often minimal (~0.1 ml), except in cases of CAVD, in which 0.3–1.0 ml may be aspirated.
- The needle is withdrawn from the epididymis and the aspirate is flushed into a 0.5–1.0 ml 37°C sperm medium.
- The tube containing the epididymal aspirate is transferred to the laboratory for microscopic examination. PESA 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 PESA fails to retrieve motile sperm for ICSI, TESA is performed at the same operative time (see Figure 5.1B).

Testicular sperm aspiration (TESA)

Indications

TESA may be performed in either OA or NOA cases. In OA, TESA is carried out after a failed PESA, but may be also used as a primary retrieval procedure in cases of absent epididymis or intense epididymal fibrosis. In NOA, TESA may be used as a diagnostic tool

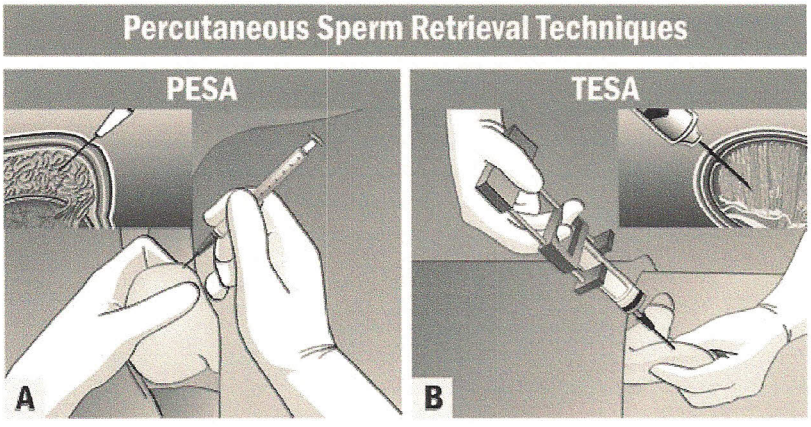

Figure 5.1. Percutaneous sperm retrieval techniques. (A) Percutaneous epididymal sperm aspiration (PESA). Epididymis is stabilized between the index finger, thumb, and forefinger. A needle attached to a tuberculin syringe is inserted into the epididymis through the scrotal skin, and fluid is aspirated. Aspirate is flushed into a tube containing HEPES-buffered sperm medium and sent for microscopic examination. (B) Testicular sperm aspiration (TESA). A 20-ml needled-syringe connected to a holder is percutaneously inserted into the testis. Negative pressure is created and the tip of the needle is moved within the testis to disrupt the seminiferous tubules and sample different areas. A piece of testicular tissue is aspirated, and a forceps is used to remove the seminiferous tubules that exteriorize from the scrotal skin. The specimen is flushed into a tube containing sperm medium, and the tube is transferred to the laboratory for processing and examination. See colour plate section.

to obtain testicular parenchyma for histology analysis and sperm search previous to the ICSI cycle. Also, it is indicated for sperm retrieval in cases of favorable prognosis, such as the ones with a previously successful TESA attempt or those with testicular biopsy result showing hypospermatogenesis.

Technique

- After anesthetic blockade of the spermatic cord, epididymis is stabilized between the index finger, thumb, and forefinger while the anterior scrotal skin is stretched.
- A 23 gauge needle attached to a 20 ml syringe is connected to a syringe holder and is inserted through the stretched scrotal skin into the anteromedial or anterolateral portion of the superior testicular pole, in an oblique angle towards the medium and lower poles (see Figure 5.1B). Loupe-magnification is used to avoid small vessels seen through the skin.
- Negative pressure is created by pulling the syringe holder while the tip of the needle is moved in and out within the testis in an oblique plane to disrupt the seminiferous tubules and sample different areas. When a small piece of testicular tissue is aspirated, the needle is gently withdrawn from the testis while the negative pressure is maintained. A pair of microsurgery forceps is used to grab the seminiferous tubules that exteriorize from the scrotal skin, thus aiding in the removal of the specimen.
- The specimen is flushed into a tube containing 0.5–1.0 ml warm sperm medium, and is transferred to the laboratory for microscopic examination. TESA or TESE may be performed at the contralateral testis if insufficient or no sperm are obtained.

Microsurgical sperm retrieval techniques

Anesthesia

Microsurgical sperm retrieval may be performed under either local anesthesia in association with intravenous sedation or epidural anesthesia. In the case of the former, which is our preference, a 10 ml solution of 2% lidocaine is injected around the spermatic cord near the external inguinal ring. Operating microscope and microsurgery technique are used throughout the procedures (see Figure 5.2).

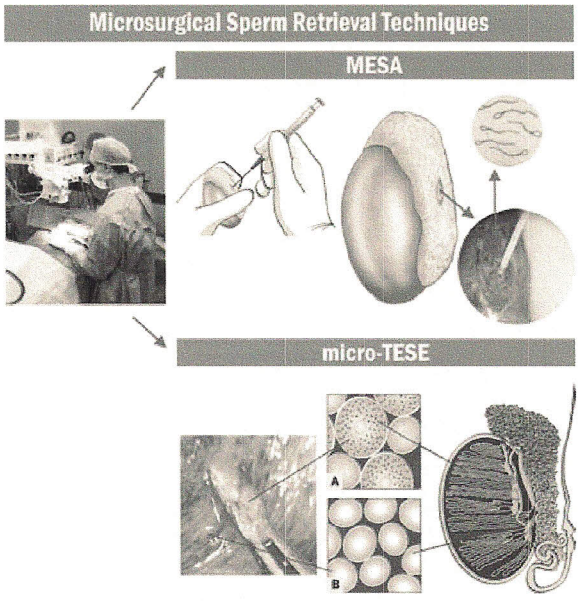

Figure 5.2. Microsurgical sperm retrieval techniques. Operating microscope and microsurgical technique are used throughout the procedures. Microsurgical epididymal sperm aspiration (MESA): after exposure of testis and epididymis, a dilated epididymal tubule is dissected and opened. Fluid is aspirated, diluted with sperm medium, and sent to the laboratory for examination. Microsurgical testicular sperm extraction (micro-TESE): after testis exteriorization, a single and large incision is made in an avascular area of the albuginea to expose testicular parenchyma. Microdissection of seminiferous tubules is carried out to identify and remove large tubules that are most likely to contain germ cells and active spermatogenesis (see photograph at ×40 magnification indicating enlarged (A) and non-enlarged (B) seminiferous tubules). Enlarged tubules may contain active spermatogenesis, as illustrated in the transversal section of a histopathology specimen (A). Non-enlarged tubules are more likely to contain no active spermatogenesis (B). Excised testicular specimens are washed in a well-dish containing sperm media to remove blood clots and are sent to the laboratory for processing and examination. See colour plate section.

Microsurgical epididymal sperm aspiration (MESA)

Indications

MESA is indicated in obstructive azoospermia cases only.

Technique

- After anesthetic blockade of spermatic cord, the anterior scrotal skin is stretched and the skin and tunica vaginalis are infiltrated with 2 ml of 2% lidocaine. A transverse 2 cm incision is made through the anesthetized layers, and the testis is exteriorized.
- The epididymis is examined and its tunica is incised. An enlarged tubule is dissected and opened with sharp microsurgical scissors.

- Fluid exuding from the tubule is aspirated with a silicone tube or blunted needle attached to a 1 ml tuberculin syringe. The aspirate is flushed into a 0.5–1.0 ml 37°C sperm medium.
- The tube containing the epididymal aspirate is transferred to the laboratory for microscopic examination. MESA 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 may be performed at the same operative time.

Microsurgical testicular sperm extraction (micro-TESE)

Indications

Micro-TESE is indicated in NOA cases only.

Technique

- After anesthetic blockade of spermatic cord, the anterior scrotal skin is stretched and the skin and tunica vaginalis are infiltrated with 2 ml of 2% lidocaine. A transverse 2 cm incision is made through the anesthetized layers, and the testis is exteriorized.
- 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 carried out at 16–25× magnification searching for enlarged seminiferous tubules (more likely to contain germ cells and eventually normal sperm production). The superficial and deep testicular regions may be examined, if necessary, and microsurgical-guided testicular biopsies are performed by removing enlarged tubules (see Figure 5.2B). If enlarged tubules are not seen, then any tubule different than the remaining ones in size is excised [10]. If all tubules are identical in appearance, random micro-biopsies (at least three at each testicular pole) are performed.
- Each excised testicular tissue specimen is placed at the outer-well 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 (TESE)

Indications

Single or multiple open testicular biopsies may be taken to obtain sperm in both OA and NOA, but TESE is used mainly in cases of NOA. In OA, TESE may be used after failed PESA or TESA. In NOA, TESE may also be used as a diagnostic tool to obtain testicular parenchyma for histology analysis and search of sperm previous to the ICSI cycle.

Anesthesia

TESE may be performed under either local anesthesia with or without intravenous sedation or epidural anesthesia.

Technique

- After anesthetic blockade of spermatic cord, the anterior scrotal skin is stretched and the skin and tunica vaginalis are infiltrated with 2 ml of 2% lidocaine. A transverse 2 cm incision is made through the anesthetized skin, cremaster, and parietal tunica vaginalis. Conventional TESE is carried out without magnification.
- A small self-retaining eyelid retractor is placed to improve exposure of the tunica albuginea, since the testis is not exteriorized.
- The tunica albuginea is incised for approximately 1 cm. Gentle pressure is made on the testis to extrude testicular parenchyma.
- A fragment of approximately 5×5×5 mm is excised with sharp scissors and placed promptly in sperm culture media (see Figure 5.3). Specimen is sent to the laboratory for processing and microscopic examination.
- Albuginea is closed using non-absorbable sutures. Procedure may be repeated if multiple biopsies are selected for preference.

Clinical outcomes

Out of 2136 males seeking infertility evaluation at our tertiary Center in Brazil from 2002 to 2009, 142 (6.6%) and 176 (8.2%) had obstructive and non-obstructive azoospermia, respectively, and underwent sperm retrieval for either diagnostic or therapeutic purposes. In this section, we present success rates of percutaneous and microsurgical techniques both in OA and NOA, and clinical outcomes of ICSI using fresh epididymal and testicular spermatozoa.

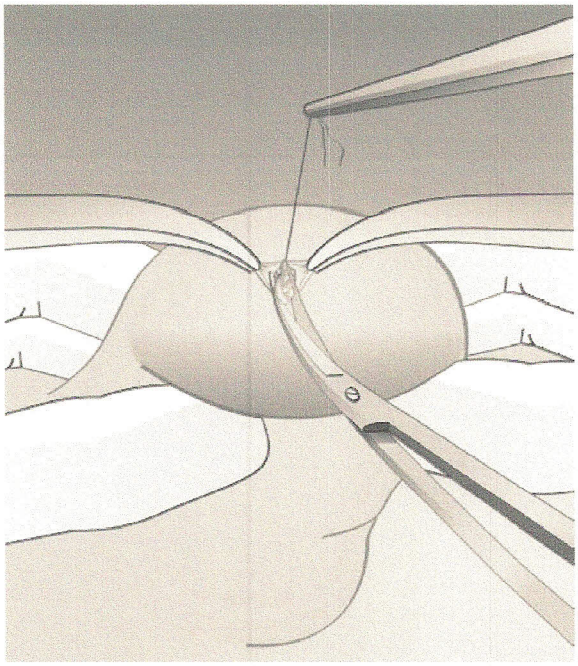

Figure 5.3. Testicular sperm extraction (TESE). Illustration of TESE using a single open biopsy. A 2-cm skin incision is made to allow opening of scrotal layers down to the albuginea. Testicle is not exteriorized from scrotum. A small 0.5-cm incision is made in an avascular area of the albuginea to expose testicular parenchyma. A fragment of approximately 5×5×5 mm is excised and sent to the laboratory for processing and examination. Additional fragments may be taken from the same incision or from different testicular poles using multiple incisions. See colour plate section.

Sperm retrieval rates

PESA and TESA were 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 several cases. In cases of failed PESA, TESA was adequate to obtain sperm in practically all cases. Motile spermatozoa was 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 (see Table 5.1). Successful sperm retrieval using percutaneous techniques appears to be independent of the cause of obstruction, since SRR rates did not differ among groups (see Table 5.1). In the group of men with NOA, SRR rates were in the range of 50–70% in most etiology-specific causes of NOA (see Table 5.2). Testicular histopathology results were predictive of sperm collection using both TESA and micro-TESE. According to our data involving 176 individuals, overall SRR rates by TESA were 64.4%, but only 20.7% and 33.3% in cases of Sertoli-cell only and maturation arrest, respectively. On the other hand, SRR by TESA was 100% and 82.3% in NOA men presenting with either hypospermatogenesis on testicular histology or a history of a previous successful TESA attempt. Using micro-TESE, overall SRR rates were 52.3%, but higher than TESA in cases of maturation arrest and Sertoli cell-only (see Table 5.2). The overall sperm retrieval rates (SRR), defined as successful surgical collection of spermatozoa, were significantly higher in the OA group (SSR = 97.9%; $n = 139/142$) compared to NOA (SSR = 61.9%; $n = 109/176$) ($P < 0.001$). The chances of retrieving spermatozoa were markedly increased in couples whose male partner had obstructive rather than non-obstructive azoospermia (OR 43.0; 95% CI 10.3–179.5).

ICSI outcomes using epididymal and testicular spermatozoa

ICSI outcomes in men with OA seem to be independent of the cause of obstruction. In the group of men with OA, fertilization and live birth rates were not different in individuals who had vasectomy / failed reversal, CBAVD, or infection as the cause of obstruction (see Table 5.3). Either epididymal or testicular spermatozoa retrieved from men with OA exhibited similar reproductive potential (see Table 5.4). Fertilization rates by ICSI using spermatozoa from men with OA and NOA were 62.5% and 51.1%, respectively ($P < 0.01$). The overall pregnancy rates, defined as the live birth rate (LBR) per transfer, were 40.2% (41/102) and 25.0% (22/88) in groups of OA and NOA, respectively ($P = 0.03$). The chances of achieving a live birth by ICSI (OR 1.93; 95% CI 1.04–3.61) were increased in couples whose male partner had obstructive rather than non-obstructive azoospermia, indicating that the reproductive potential of infertile men undergoing ART is related to the type of azoospermia.

Expert commentary

Obstructive azoospermia

The adoption of strict criteria to diagnose OA is crucial for obtaining high success retrieval rates in the

Table 5.1. Sperm retrieval rates (SRR) of PESA and TESA in obstructive azoospermia (AO) according to the number of aspiration attempts and the cause of obstruction

	Presence of Motile sperm by PESA: *n* (%)	Cumulative Successful retrieval rate: *n* (%)
By retrieval attempt: *n* (%) – 130 (100.0%)		
1st PESA attempt	66/130 (50.8)	–
2nd PESA attempt	29/64 (45.3)	95/130 (73.1)
3rd PESA attempt	10/35 (28.6)	105/130 (80.7)
4th PESA attempt	7/25 (28.0)	112/130 (86.1)
Rescue TESA after failed PESA	16/18 (88.9)	128/130 (98.4)
By cause of obstruction: *n* (%) – 142 (100.0%)[a]		
CAVD – 30 (23.1%)	21/30 (70.0)	30/30 (100.0)[a]
Vasectomy/failed reversal – 64 (49.2%)	40/64 (62.5)	61/64 (95.3)[a]
Post-infection/EDO/trauma – 48 (36.9%)	31/36 (81.6)	48/48 (100.0)[a]
Overall SRR: *n* (%)	92/130 (70.8)	139/142 (97.9)[a]

Source: Androfert.
CAVD, congenital absence of vas deferens; EDO, ejaculatory duct obstruction.
[a] PESA+TESA: in 12 cases of post-infectious OA, TESA was performed as the first choice due to intense epididymis fibrosis. In three of them, no sperm was obtained after TESA.
Chi-square test was used to compare SRR rates among groups stratified by the cause of obstruction. Results were not significantly different; $P < 0.05$ considered significant.

Table 5.2. Sperm retrieval rates (SRR) in 176 non-obstructive azoospermic men according to the surgical method of collection stratified by histopathology results and cause of azoospermia

	Presence of testicular spermatozoa: *n* (%)
By method: *n* = 176 (100.0%)	
TESA (*n* = 90); overall SRR, *n* (%)	58/90 (64.4)
Hypospermatogenesis	26/26 (100.0)
Maturation arrest	2/6 (33.3)
Sertoli-cell only	6/29 (20.7)
Not available[a]	24/29 (82.3)
Micro-TESE (*n* = 86); overall SRR, *n* (%)	45/86 (52.3)
Hypospermatogenesis	19/19 (100.0)
Maturation arrest	7/12 (58.3)
Sertoli-cell only	13/39 (33.3)
Not available	6/16 (37.5)
By cause of NOA: *n* = 176 (100.0%)	
Varicocele (*n* = 66)	45/66 (68.2%)
Genetic (*n* = 12)[b]	6/12 (50.0%)
Cryptorchidism (*n* = 19)	12/19 (63.1%)
Idiopathic (*n* = 63)	33/63 (52.4%)
Radiotherapy/chemotherapy (*n* = 6)	3/6 (50.0%)
Orchitis/gonadotoxin/endocrine (*n* = 10)	10/10 (100.0%)
Overall SRR: *n* (%)	109/176 (61.9)

Source: Androfert.
[a] Cases with previous successful TESA attempt.
[b] Includes cases of Klinefelter syndrome and AZFc Y-chromosome microdeletions.

range of 90–100% using percutaneous techniques. Using PESA, our approach is to perform the first 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. Most cases of PESA failures are not necessarily technical failures because immotile spermatozoa are found. However, in certain cases of epididymal fibrosis due to multiple PESA attempts or post-infection, PESA may be ineffective to retrieve sperm. In these cases, PESA can be attempted at the contralateral epididymis or TESA can be applied successfully if there is spermatogenesis in the testes. Routinely, procedures are performed under local anesthesia, with or without intravenous sedation. Percutaneous sperm retrieval techniques can be performed both for diagnostic and for therapeutic purposes. In the latter, sperm retrieval is often carried out on the same day as oocyte retrieval or the day before. Patients are discharged one hour later and can return to normal activities the same day. Oral analgesics are prescribed but pain complaints are minimal. The most common complication is fibrosis at the aspiration site. Other potential complications include hematoma, bleeding, and infection, but they are rare [6]. Some authors claim that MESA allows the collection of larger and cleaner quantities of sperm than PESA [2], but this debate seems trivial.

Table 5.3. ICSI outcomes according to the cause of obstructive azoospermia (AO)

	CAVD	Vasectomy/failed reversal	Infection/other
Number of cycles: $n = 145$	32	59	54
Female age in years: mean ± SD	31.4±5.0	32.6±6.2	32.9±5.9
2PN fertilization rate: mean (%)	64.1%	65.3%	59.3%
Cleavage rate: mean (%)	98.9%	98.8%	99.1%
Top-quality embryo for transfer: mean[a] (%)	44.9%	57.9%	49.4%
Number of embryos transferred: mean	2.9	2.6	3.0
Clinical pregnancy rate per transfer: *n* (%)	16/29 (55.2%)	26/59 (44.0%)	23/53 (43.4%)
Miscarriage rate: *n* (%)	5/16 (31.2%)	7/26 (26.7%)	3/23 (13.1%)
Live birth rate per transfer: *n* (%)	11/29 (37.8%)	19/59 (32.2%)	20/53 (37.7%)

Source: Androfert.
CAVD, congenital absence of vas deferens.
[a] 7–9 blastomeres of similar size, and grades I or II cytoplasmic fragmentation on the day of embryo transfer (day 3).
One-way ANOVA and Chi-square test were used to compare laboratory and clinical parameters among groups. Results were not significantly different; $P < 0.01$ considered significant.

Table 5.4. ICSI outcomes using spermatozoa retrieved from men with obstructive (AO) and non-obstructive azoospermia (NOA)

	Obstructive azoospermia		Non-obstructive azoospermia
Source of sperm for ICSI	**Epididymal**	**Testicular**	**Testicular**
Number of cycles: $n = 107$	93	14	95
Female age in years: mean ± SD	32.6 ± 5.3	32.1 ± 5.4	33.1 ± 5.7
2PN fertilization rate: %	66.0[b]	56.6[b]	51.1[c]
Cleavage rate: %	99.4	95.7	96.7
Top-quality embryo rate for transfer:[a] %	51.9	48.9	46.1
Cycles with embryo transfer: *n* (%)	88 (94.6)	14 (100.0)	88 (92.6%)
Number of embryos transferred: mean	2.8	3.3	2.7
Clinical pregnancy rate per transfer: *n* (%)	45/88 (51.1)[d]	7/14 (50.0)[d]	28/88 (31.8)[e]
Miscarriage rate: *n* (%)	11/45 (24.4)	1/7 (14.3)	6/28 (21.4)
Live birth rate per transfer: *n* (%)	34/88 (38.6)[f]	6/14 (42.8)[f]	22/88 (25.0)[g]

Source: Androfert.
Values expressed as means for fertilization, cleavage, and embryo quality rates.
[a] 7–9 blastomeres of similar size, and grades I or II cytoplasmic fragmentation on the day of embryo transfer (day 3).
One-way ANOVA and Chi-square test were used to compare laboratory and clinical ICSI parameters between OA and NOA groups, and between epididymal and testicular sperm in OA group. Statistically significant results were obtained only for fertilization ([b×c]$P < 0.001$), clinical pregnancy ([d×e]$P = 0.008$), and live birth rates ([f×g]$P = 0.03$) between NOA and OA groups; $P < 0.05$ considered significant.

In our series of 142 men with OA, the cumulative successful retrieval rate after PESA and/or TESA was higher than 95%, and an adequate number of motile sperm for cryopreservation was obtained in approximately one-third of the cases (35/112). Clinical outcomes of ICSI using PESA or TESA-derived spermatozoa were not different, indicating that sperm fertility potential is independent of the source in OA. Moreover, we have demonstrated that ICSI outcomes using fresh epididymal and testicular

spermatozoa retrieved from men with OA are comparable to those obtained with ejaculated sperm [13]. Although the cryopreservation rate after PESA is not high, repeated aspirations can be carried out in men with OA with minimal morbidity and lower cost compared to MESA. In rare circumstances, we perform MESA for sperm retrieval in OA men with coagulation disorders.

Non-obstructive azoospermia

The best sperm retrieval technique in NOA is yet to be established. To date, no randomized controlled trial has compared the efficiency of these strategies and thus current recommendations are based on cumulative evidence provided by descriptive, observational, and controlled studies. The efficiency of TESA for retrieving spermatozoa in NOA varies from 10% to 30% [14], except in the favorable cases of men with previous successful TESA or testicular histopathology showing hypospermatogenesis. In such individuals, SRR rates by TESA are in the range of 70–100% [12, 15, 16]. In a recent systematic review the mean reported SRR for TESE was 49.5%. TESE with multiple biopsies resulted in higher SRR than fine-needle aspiration, a variation of TESA, especially in cases of Sertoli-cell-only (SCO) syndrome and maturation arrest [17]. In NOA, current evidence suggests that micro-TESE performs better than conventional TESE or TESA in cases of SCO, where tubules containing an active focus of spermatogenesis can be identified. Micro-TESE also appears to be the safest technique regarding postoperative complications. Proper identification of testicular vessels under the tunica albuginea is made prior to the placement of an incision into the testis. The use of optical magnification and microsurgery techniques allows the preservation of intratesticular blood supply, as well as the identification of tubules more likely to harbor sperm production [10–12, 18]. Therefore, the efficacy of sperm retrieval is improved while the risks of large tissue removal are minimized. Excision of large biopsy samples in conventional TESE has been shown to impair testosterone production [18]. Tissue removal in micro-TESE is often 50–70-fold less than conventional TESE [8–10], and the small amount of tissue extracted facilitates sperm processing.

The clinical outcomes of ICSI using testicular sperm extracted by TESA or micro-TESE in NOA are significantly lower than those obtained with either ejaculated or epididymal/testicular sperm from men with OA [13]. From the limited data available, it is suggested that the sperm retrieval technique itself has no impact on ICSI success rates [17]. Our data indicate that testicular spermatozoa of men with severely impaired spermatogenesis have decreased fertility potential, and may have a higher tendency to carry deficiencies such as the ones related to the centrioles and genetic material, which ultimately affect the capability of the male gamete to activate the egg and trigger the formation and development of a normal zygote and a viable embryo [13].

Predictive factors for retrieving sperm in non-obstructive azoospermic men

Testicular spermatozoa can be obtained in most etiology-specific causes of NOA, such as varicocele, cryptorchidism, orchitis, and genetic, endocrine, and gonadotoxic-induced cases [5, 10, 19–24]. In genetic-related NOA, such as Y-chromosome infertility and Klinefelter syndrome (KS), pregnancies may be achieved by ICSI in males with retrievable testicular sperm [22–24]. The presence or absence of retrievable sperm in azoospermic men with Y-chromosome infertility varies depending on the specific microdeletion. In partial and complete AZFc deletion azoospermic patients, testicular sperm can be found in approximately 70% of the cases. In contrast, the chance of finding sperm in azoospermic men with complete AZFa or AZFb deletions is unlikely [22]. If a successful pregnancy is obtained, male offspring will harbor the same deletion as their father, with a high risk of male infertility. In NOA men with KS, sperm are found in approximately 50% of the cases on testicular exploration. Pregnancy rates by ICSI range from 30% to 50% and children who have been born have a normal karyotype [23]. It has been recently demonstrated that germ cells in men with KS are euploid, 46,XY, and thus can form normal, haploid gametes [24].

Although not absolute, testicular histology is still considered the best predictor for successful sperm retrieval in NOA men. The probability of obtaining sperm varies according to the testicular histopathology results [12, 16], as also shown by our own data (Table 5.2). However, even the combination of histology and FSH levels provides only a "fair" prediction model for sperm retrieval (accuracy of 0.74) [25], and

testicular sperm can be collected even in the more adverse histopathology pattern. FSH levels have also been used as a marker of testicular reserve, but it has been recently demonstrated that normal FSH levels in NOA men are not predictive of SRR [11, 26]. Serum FSH reflects the global spermatogenic function, but in cases of diffuse maturation arrest, adequate control feedback from germ cells and Sertoli cells exists despite the absence of sperm production [26]. Sperm can be retrieved from testicles of men with elevated serum FSH, and SRR rates appear to be correlated with the technique of sperm retrieval rather than with FSH levels. Significantly higher retrieval rates (~60%) were reported using micro-TESE compared to random multiple testicular biopsies in NOA men with elevated FSH levels [11, 27].

The importance of surgical and medical treatment prior to sperm retrieval in NOA men has been recently highlighted. It has been suggested that treatment of clinical varicoceles prior to sperm retrieval significantly increased the chance of testicular sperm collection by micro-TESE in a group of NOA individuals with clinical varicoceles [19]. In this retrospective study, SRR rates were 53% and 30% in the treated and untreated men, respectively (OR: 2.63; 95% CI 1.05–6.60, $P = 0.03$). Medical therapy (aromatase inhibitors, clomiphene, or human chorionic gonadotropin) prior to micro-TESE was also shown to enhance sperm retrieval success rates in Klinefelter syndrome men who responded to medication by increasing serum testosterone to more than 100 ng/dL from baseline [28].

Tips and pitfalls

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. On 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 alleviating 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. TESE 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. TESE/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 hCG administration to microinjection does not exceed 44 h [29]. 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.

The concept of cryopreservation may be used in association with sperm retrieval procedures. Epididymal and testicular spermatozoa can be cryopreserved using protocols routinely used for ejaculated sperm [30, 31]. Some centers prefer to retrieve and intentionally cryopreserve sperm for future use. This strategy offers the advantage of avoiding ovarian stimulation when no sperm is obtained from testicular specimens. If sperm is found and frozen, thawing can be done at any time, thus obviating the need to organize two operations (oocyte and sperm retrieval) on the same day. Also, cryopreservation may be an interesting tool to preserve left-over specimens that would be discharged after ICSI, especially if the treatment cycle does not result in a pregnancy. Future ICSI attempts may be carried out without repeated surgical retrievals. 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 seem not to differ [16, 30, 31]. If only immotile spermatozoa are obtained, a method for selecting viable sperm for ICSI may be used, since it has been observed that conventional seminal parameters have little or no influence in ICSI outcomes, except when only immotile spermatozoa are available [32]. Methods for selecting immotile viable sperm for ICSI, such as hyposmotic swelling [33, 34], sperm tail flexibility [35, 36], or motility stimulant sperm challenge tests [37–40] are available, but results are limited for cryopreserved specimens. 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 poorer than with fresh ones [31]. 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 frozen specimens would be preferable. If not, fresh specimens are preferable. Currently, our cryopreservation technique for surgically retrieved sperm is the standard liquid nitrogen vapor method using TEST-yolk buffer and glycerol as cryoprotectants [41, 42]. Epididymal specimens are concentrated by washing before freezing, and testicular sperm are freed from the testicular parenchyma, i.e. testicular homogenates are frozen. Recently, it has been demonstrated that human spermatozoa can be successfully vitrified, and this strategy may be of interest for preserving small quantities of surgically retrieved gametes [43].

Conclusions and key points

In OA, sperm production is normal and gametes can be easily retrieved from epididymis or testicle in most cases, irrespective of the technique. PESA or TESA are simple and efficient methods for retrieving epididymal or testicular spermatozoa in men with OA. For NOA, TESE with or without magnification is the preferred approach, and sperm can be retrieved in approximately 60% of the cases. The use of microsurgery during TESE may improve the efficacy of sperm extraction with significantly less tissue removed, which ultimately facilitates sperm processing. Testicular histology results, if available, may be useful to predict the chances of retrieving sperm in men with NOA. However, sperm can be obtained even in the worst-case scenario except in cases of Y chromosome infertility with complete AZFa and/or AZFb microdeletions. In both OA and NOA, the sperm retrieval technique itself seems to have no impact on ICSI success rates. The main goal of PESA/TESA/TESE sperm processing is the recovery of a clean sample containing motile sperm. Such specimens are more fragile, and often compromised in motility, as compared to those obtained from ejaculates. Laboratory techniques should be carried out with great caution not to jeopardize the sperm fertilizing potential. Surgically retrieved spermatozoa can be intentionally cryopreserved for future use. Spare left-over specimens that would be discharged after ICSI can also be cryostored. Different strategies can be developed according to each group's results. If freezing of surgically retrieved specimens provides results similar to those with the use of fresh sperm, then the use of frozen specimens would be preferable. If not, fresh specimens are preferable. The reproductive potential of infertile men undergoing ART 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 has obstructive rather than non-obstructive azoospermia.

Glossary

Azoospermia.	Absence of spermatozoa in the microscopic examination of the seminal fluid after centrifugation on at least two separate occasions.
Cryopreservation.	The freezing process for storage of gametes or gonadal tissue at ultra-low temperature.
ICSI.	Intracytoplasmic sperm injection: a procedure in which a single spermatozoon is injected into the oocyte cytoplasm.
MESA.	Microsurgical epididymal sperm aspiration: a microsurgical procedure used to aspirate spermatozoa directly from the epididymal tubules for use in an ICSI procedure.

Micro-TESE.	Microdissection testicular sperm extraction: a microsurgical procedure used to dissect the seminiferous tubules within the testis in an attempt to identify areas of sperm production and extract spermatozoa for use in an ICSI procedure.
PESA.	Percutaneous epididymal sperm aspiration: a procedure in which a needle is inserted into the epididymis to retrieve spermatozoa for use in an ICSI. procedure.
Sperm processing.	Laboratory techniques used to remove contaminants (cellular debris, microorganisms, red blood cells, etc.) and to select the best-quality spermatozoa to be used in conjunction with assisted reproduction technology.
TESA.	Testicular sperm aspiration: a procedure in which a needle is inserted into the testicle in order to retrieve spermatozoa for use in an ICSI procedure.
TESE.	Testicular sperm extraction: operative removal of testicular tissue in an attempt to collect sperm for use in an ICSI procedure.

Acknowledgments

The authors would like to acknowledge Sidney Verza Jr., Christina Prudencio and Bill Seol for their assistance in data collection, and Mrs. Fabiola Bento for editorial and text revision.

References

1. Palermo G, Joris H, Devroey P, *et al.* Pregnancies after intracytoplasmic sperm injection of single spermatozoan into an oocyte. *Lancet* 1992;**340**:17–18.
2. Silber S, Nagy ZP, Liu J, *et al.* Conventional in-vitro fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration. *Hum Reprod* 1994;**9**:1705–9.
3. Devroey P, Liu J, Nagy ZP, *et al.* Pregnancies after testicular extraction (TESE) and intracytoplasmic sperm injection (ICSI) in non-obstructive azoospermia. *Hum Reprod* 1995;**10**:1457–60.
4. Esteves SC. Infertilidade masculina. In: Rhoden EL, ed. *Urologia no consultório*, 1st edn. Porto Alegre: Artmed Editora; 2009: 470–500.
5. Esteves SC, Glina S. Recovery of spermatogenesis after microsurgical subinguinal varicocele repair in azoospermic men based on testicular histology. *Int Braz J Urol* 2005;**31**:541–8.
6. Craft I, Tsirigotis M, Bennett V, *et al.* Percutaneous epididymal sperm aspiration and intracytoplasmic sperm injection in the management of infertility due to obstructive azoospermia. *Fertil Steril* 1995;**63**:1038–42.
7. Craft I, Tsirigotis M. Simplified recovery, preparation and cryopreservation of testicular spermatozoa. *Hum Reprod* 1995;**10**:1623–7.
8. Okada H, Dobashi M, Yamazaki T, *et al.* Conventional versus microdissection testicular sperm extraction for nonobstructive azoospermia. *J Urol* 2002; **168**:1063–7.
9. Tsujimura A, Matsumiya K, Miyagawa Y, *et al.* Conventional multiple or microdissection testicular sperm extraction: a comparative study. *Hum Reprod* 2002;**17**:2924–9.
10. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Hum Reprod* 1999;**14**:131–5.
11. Ramasamy R, Lin K, Gosden LV, *et al.* High serum FSH levels in men with nonobstructive azoospermia does not affect success of microdissection testicular sperm extraction. *Fertil Steril* 2009;**92**:590–3.
12. Esteves SC, Verza S Jr, Gomes AP. Successful retrieval of testicular spermatozoa by microdissection (micro-TESE) in nonobstructive azoospermia is related to testicular histology. *Fertil Steril* 2006;**86**:S354.
13. Verza S Jr, Esteves SC. Sperm defect severity rather than sperm source is associated with lower fertilization rates after intracytoplasmic sperm injection. *Int Braz J Urol* 2008, **34**: 49–56.
14. Friedler S, Raziel A, Strassburger D, *et al.* Testicular sperm retrieval by percutaneous fine needle aspiration compared to testicular sperm extraction by open biopsy in men with non-obstructive azoospermia. *Hum Reprod* 1997;**12**:1488–93.
15. Fasouliotis SJ, Safran A, Porat-Katz A, *et al.* A high predictive value of the first testicular fine needle aspiration in patients with non-obstructive azoospermia for sperm recovery at the subsequent attempt. *Hum Reprod* 2002;**17**:139–42.

16. Sousa M, Cremades N, Silva J, *et al.* Predictive value of testicular histology in secretory azoospermic groups and clinical outcome after microinjection of fresh and frozen-thawed sperm and spermatids. *Hum Reprod* 2002; **17**:1800–10.

17. Donoso P, Tournaye H, Devroey P. Which is the best sperm retrieval technique for non-obstructive azoospermia? A systematic review. *Hum Reprod Update* 2007;**13**:539–49.

18. Schlegel PN, Su LM. Physiologic consequences of testicular sperm extraction. *Hum Reprod* 1997;**12**:1688–92.

19. Inci K, Hascicek M, Kara O, *et al.* Sperm retrieval and intracytoplasmic sperm injection in men with nonobstructive azoospermia, and treated and untreated varicocele. *J Urol* 2009;**182**:1500–5.

20. Chan PT, Palermo GD, Veeck LL, *et al.* Testicular sperm extraction combined with intracytoplasmic sperm injection in the treatment of men with persistent azoospermia postchemotherapy. *Cancer* 2001; **92**:1632–7.

21. Fahmy I, Kamal A, Shamloul R, *et al.* ICSI using testicular sperm in male hypogonadotrophic hypogonadism unresponsive to gonadotrophin therapy. *Hum Reprod* 2004;**19**:1558–61.

22. Hopps CV, Mielnik A, Goldstein M, *et al.* Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. *Hum Reprod* 2003;**18**:1660–5.

23. Schiff JD, Palermo GD, Veeck LL, *et al.* Success of testicular sperm injection and intracytoplasmic sperm injection in men with Klinefelter syndrome. *J Clin Endocrinol Metab* 2005;**90**:6263–7.

24. Sciurano RB, Luna Hisano CV, Rahn MI, *et al.* Focal spermatogenesis originates in euploid germ cells in classical Klinefelter patients. *Hum Reprod* 2009;**24**:2353–60.

25. Tournaye H, Verheyen G, Nagy ZP, *et al.* Are there any predictive factors for successful testicular sperm recovery in azoospermic patients? *Hum Reprod* 1997;**12**:80–6.

26. Hung AJ, King P, Schlegel PN. Uniform testicular maturation arrest: a unique subset of men with nonobstructive azoospermia. *J Urol* 2007;**178**:608–12.

27. Bromage SJ, Falconer DA, Lieberman BA, *et al.* Sperm retrieval rates in subgroups of primary azoospermic males. *Eur Urol* 2007;**51**:534–9.

28. Ramasamy R, Ricci JA, Palermo GD, *et al.* Successful fertility treatment for Klinefelter's syndrome. *J Urol* 2009;**182**:1108–13.

29. Schneider DT, Gomes AP, Verza S Jr, *et al.* Optimal time interval for intracytoplasmic sperm injection after administration of human chorionic gonadotrophin in severe male factor infertility. *Fertil Steril* 2006;**86**:S155.

30. Devroey P, Silber S, Nagy ZP, *et al.* Ongoing pregnancies and birth after intracytoplasmic sperm injection with frozen-thawed epididymal spermatozoa. *Hum Reprod* 1995;**10**:903–6.

31. Fisher R, Baukloh V, Naether GJO, *et al.* Pregnancy after intracytoplasmic sperm injection of spermatozoa from frozen-thawed testicular biopsy. *Hum Reprod* 1996;**11**:2197–9.

32. Nagy ZP, Liu J, Joris H, *et al.* The result of intracytoplasmic sperm injection is not related to any of the three basic sperm parameters. *Hum Reprod* 1995;**10**:1123–9.

33. Esteves SC, Sharma RK, Thomas A Jr, *et al.* Suitability of the hypo-osmotic swelling test for assessing the viability of cryopreserved sperm. *Fertil Steril* 1996;**66**:798–804.

34. Sallam HN, Farrag A, Agameya A-F, *et al.* The use of the modified hypo-osmotic swelling test for the selection of immotile testicular spermatozoa in patients treated with ICSI: a randomized controlled study. *Hum Reprod* 2005;**20**:3435–40.

35. Soares JB, Glina S, Antunes N Jr, *et al.* Sperm tail flexibility test: a simple test for selecting viable spermatozoa for intracytoplasmic sperm injection from semen samples without motile spermatozoa. *Rev Hosp Clin Fac Med Sao Paulo* 2003; **58**:250–3.

36. Oliveira NM, Sanchez RV, Fiesta SR, *et al.* Pregnancy with frozen-thawed and fresh testicular biopsy after motile and immotile sperm microinjection, using the mechanical touch technique to assess viability. *Hum Reprod* 2004;**19**:262–5.

37. Esteves SC, Sharma RK, Thomas AJ Jr, *et al.* Cryopreservation of human spermatozoa with pentoxifylline improves the post-thaw agonist-induced acrosome reaction rate. *Hum Reprod* 1998;**13**:3384–9.

38. Terriou P, Hans E, Giorgetti C, *et al.* Pentoxifylline initiates motility in spontaneously immotile epididymal and testicular spermatozoa and allows normal fertilization, pregnancy, and birth after intracytoplasmic sperm injection. *J Assist Reprod Genet* 2000;**17**:194–9.

39. Kovacic B, Vlaisavljevic V, Reljic M. Clinical use of pentoxifylline for activation of immotile testicular sperm before ICSI in patients with azoospermia. *J Androl* 2006;**27**:45–52.

40. Yovich JL. Pentoxifylline: actions and applications in assisted reproduction. *Hum Reprod* 1993;**8**:1786–91.

41. Esteves SC, Spaine DM, Cedenho AP. Effects of pentoxifylline treatment before freezing on motility, viability and acrosome status of poor quality human spermatozoa cryopreserved by the liquid nitrogen vapor method. *Braz J Med Biol Res* 2007;**40**:985–92.

42. Verza S Jr, Feijo CM, Esteves SC. Resistance of human spermatozoa to cryoinjury in repeated cycles of thaw-refreezing. *Int Braz J Urol* 2009;**35**:581–91.

43. Isachenko E, Isachenko V, Weiss JM, *et al.* Acrosomal status and mitochondrial activity of human spermatozoa vitrified with sucrose. *Reproduction* 2008;**136**:167–73.