Sperm Retrieval Techniques

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Key Points

- Percutaneous epididymal sperm aspiration (PESA) and microsurgical epididymal sperm aspiration (MESA) are the most commonly used methods to harvest epididymal sperm.
- Testicular sperm aspiration (TESA) and open testicular sperm extraction with or without the aid of microsurgery (micro-TESE and TESE, respectively) are the methods used to retrieve testicular sperm.
- Surgical sperm retrieval can be performed on an outpatient basis with the intention to cryopreserve sperm for future use or in association with oocyte retrieval and immediate sperm injection.
- In men with obstructive azoospermia, spermatogenesis is normal, and sperm can be easily retrieved from the epididymis or testis.
- In obstructive azoospermia, the sperm retrieval technique and the cause of obstruction seem to have little effect on sperm retrieval rates and ICSI outcomes. Likewise, the sperm source (epididymis or testis) and the sperm status (fresh or frozen-thawed) do not seem to impact ICSI outcomes. However, MESA yields a higher number of motile sperm than does PESA, thereby offering the possibility of cryopreserving larger quantities of sperm that might enable multiple ICSI cycles without the need for repeat sperm retrieval.
- In men with nonobstructive azoospermia, success in harvesting sperm is higher, and complication rates are lower with micro-TESE than with conventional TESE. Nevertheless, the likelihood of harvesting sperm with both approaches is related to testicular histology. Men with hypospermatogenesis and maturation arrest have a more favorable outcome than those with Sertoli cell-only syndrome.
- Testicular parenchyma or epididymal aspirates should be carefully handled in the IVF laboratory, as these specimens might be more fragile than ejaculated counterparts.
- The classification of azoospermia into obstructive azoospermia and nonobstructive azoospermia has a significant influence on sperm retrieval rates and ICSI success. Results are less favorable among men with nonobstructive azoospermia than with obstructive azoospermia.
- In non-azoospermic men with high sperm DNA fragmentation in the semen, ICSI with sperm harvested from the seminiferous tubules seems to yield better ICSI outcomes and higher live birth rates.
- The underlying parental infertility seems to have a significant effect on the health of ICSI offspring. While the risks of congenital malformations, epigenetic disorders, chromosomal abnormalities, subfertility, cancer, delayed psychological and neurological development, and impaired cardiometabolic profile are reported to be greater in infants born as a result of ICSI than in naturally conceived children, it remains to be determined to what extent the observed adverse outcomes might be aggravated by using surgically retrieved sperm.

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50.1 Introduction

Overall, 3–12% of men at reproductive age present fertility-related issues, making male infertility the sole responsible for approximately one-third of all infertility cases [1].

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Azoospermia – a complete absence of spermatozoa in the ejaculate – affects approximately 1% of the general male population and 10% of men with infertility. This condition relates to the complete absence of any sperm in the ejaculate even after centrifugation [2].

Sperm retrieval techniques were initially developed to overcome obstructive causes of male infertility associated with azoospermia, either acquired (e.g., vasectomy) or congenital (e.g., congenital bilateral absence of the vas deferens (CBAVD)). Its first successful report resulting in a pregnancy was published in 1985 when Temple-Smith and colleagues described the microsurgical epididymal sperm aspiration (MESA) [3].

Later, in 1994, Devroey and colleagues described the use of testicular sperm extracted by open biopsy (testicular sperm extraction (TESE)) to treat men who had previously failed epididymal sperm aspiration (percutaneous epididymal sperm aspiration (PESA)) [4]. Nonetheless, the first series to describe successful pregnancies using aspirated epididymal sperm was reported by Craft and Shrivastav in the same year [5]. In 1996, Lewin et al. were able to achieve pregnancy using aspirated testicular spermatozoa (TESA) to treat a nonobstructive azoospermic patient with a histological diagnosis of maturation arrest [6]. More recently, in 1999, Schlegel described the microdissection testicular sperm extraction technique (micro-TESE) which consists of direct identification of active spermatogenic regions within the testis under microscopic magnification [7]. This approach is the method of choice for sperm extraction in cases of nonobstructive azoospermia (NOA) as it offers significantly higher rates for sperm yield when compared with open TESE.

The reproductive urologist who performs sperm extraction should be familiar with all methods to recommend the best technique for each case scenario and to provide the in vitro fertilization (IVF) laboratory the best specimen for use with assisted reproductive technology (ART). One must also be able to both execute and foresee the need for a rescue procedure such as in cases of failed PESA in obstructive azoospermia (OA) which may require a rescue TESA or TESE or in cases of a misdiagnosed NOA, which may require immediate micro-TESE instead of PESA/MESA.

Knowing the surgical complications, the IVF outcomes, and the predictors of success will help the urologist to adequately counsel the patient regarding the procedure itself and its expectations toward the treatment as a whole. This knowledge will likely enhance the patient's confidence and engagement in the therapeutic proposal.

50.2 Sperm Retrieval Techniques

50.2.1 Percutaneous

50.2.1.1 Epididymal Sperm Aspiration (PESA)

Percutaneous epididymal sperm aspiration (PESA) is used to harvest epididymal sperm in cases of OA [8]. PESA is a noncomplex and straightforward method that can be performed under intravenous (IV) sedation with propofol without the need for locoregional anesthetic blockade (Fig. 50.1). A 10-mL solution of 2% lidocaine without epinephrine is injected outside the outer ring to block the spermatic cord. We use loupe magnification to avoid injury to small scrotal vessels seen through the skin [9]. A 29–33-Gauge butterfly needle was initially used to retrieve sperm from the epididymis [5]. Alternatively, nowadays it is more common to use a 23-Gauge needle connected to a 1-mL tuberculin syringe that is inserted through the skin into the epididymis with negative pressure [10]. A gentle movement in and out of the epididymis allows the aspiration of a small quantity of fluid while holding the epididymis firmly with the other hand.

PESA may be repeated at a different portion from the tail/body toward the caput epididymis until enough motile sperm is recovered for intracytoplasmic sperm injection (ICSI) or cryopreservation. TESA should be done as a rescue procedure when no motile sperm is available for ICSI [11]. A short movie depicting the main steps of the procedure can be found at http://www.brazjurol.com.br/videos/july_august_2015/Esteves_817_818video.htm [9].

50.2.1.2 Testicular Sperm Aspiration (TESA)

TESA is performed on an outpatient basis, either concomitantly with oocyte retrieval to allow immediate use of sperm for ICSI or to freeze sperm for future use (Fig. 50.2) [10]. TESA has been used to retrieve sperm from men with OA, selected cases of NOA, and, more recently, nonazoospermic men with excessive elevated DNA fragmentation [11–13]. Occasionally, the procedure is also used to obtain additional sperm in cases of cryptozoospermia when ejaculated semen is inadequate or insufficient for ICSI [14].

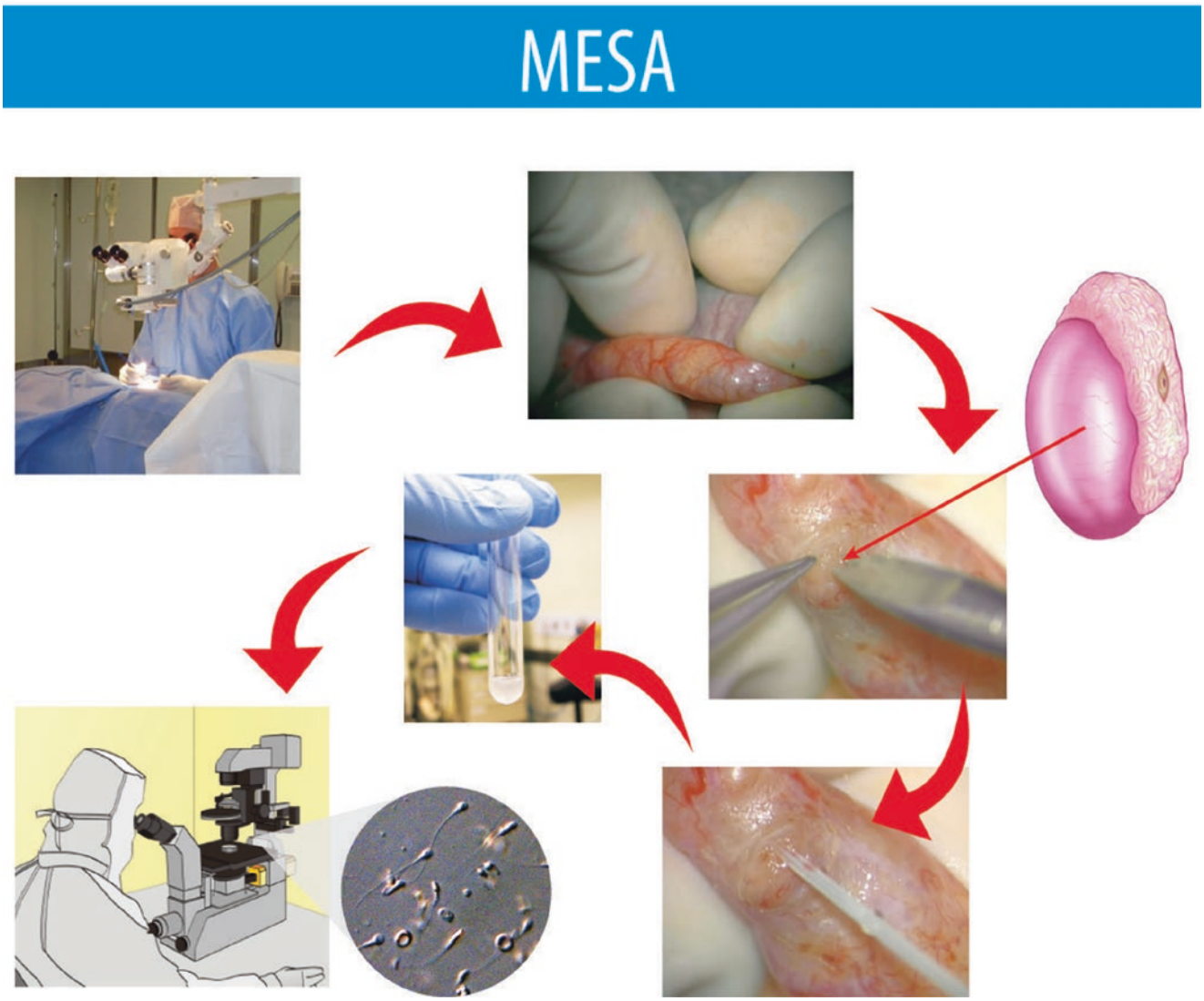

Fig. 50.1 Microsurgical epididymal sperm aspiration (MESA). A dilated epididymal tubule is dissected and cut open under surgical microscopy and microsurgical technique. Seminal fluid is aspirated, diluted with sperm medium, and sent to the laboratory for analysis (Reprinted with permission from ANDROFERT. All Rights Reserved)

TESA can be carried out under intravenous sedation or general anesthesia combined with local anesthesia applied to the spermatic cord. The testicle is held firmly and punctured using an 18-Gauge needle attached to a 20-mL syringe. Negative pressure is applied using a syringe holder (e.g., Cameco syringe holder) that aids in removal of seminiferous tubules. Like in PESA, loupe magnification may be used during puncture to avoid scrotal skin vascular injury. The needle is moved back and forth to disrupt tubules, so they can be adequately aspirated. Ideally, all testicular regions should be sampled during aspiration; the needle is inserted in an oblique angle in its anterior aspect of the upper pole. The sample is immediately analyzed in the IVF laboratory, and if inadequate, the contralateral testis is punctured at the same operative time [15]. A short movie depicting the main steps of the procedure can be found at https://www.youtube.com/watch?v=o9MgknYEzN0.

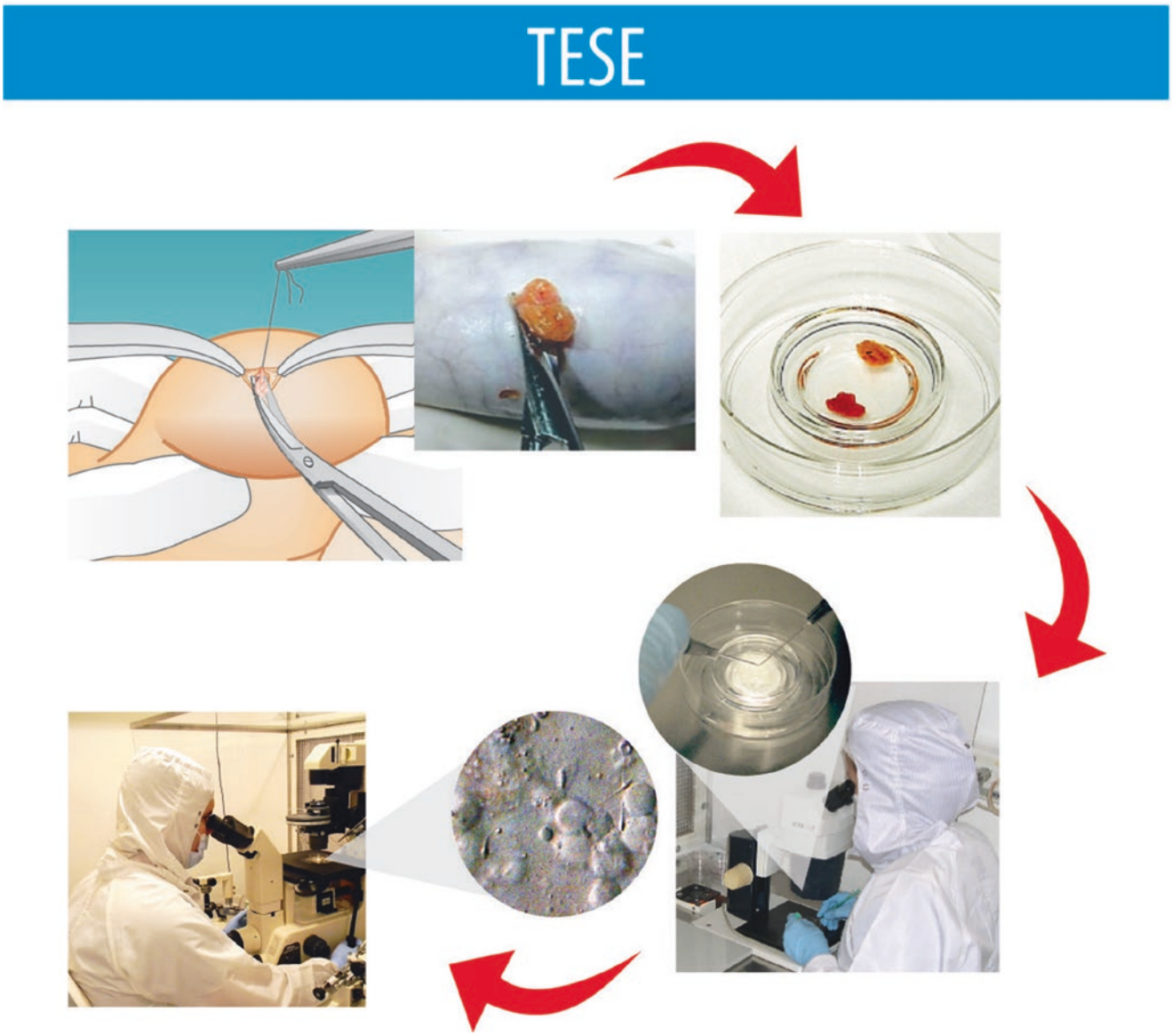

Fig. 50.2 Testicular sperm extraction (TESE). A single or multiple incisions are made on the tunica albuginea, and one or several testicular biopsies are taken. Specimens are sent to the laboratory for mechanical mincing and examination under the inverted microscope for sperm search (Reprinted with permission from ANDROFERT. All Rights Reserved)

50.2.2 Open Non-microsurgical

50.2.2.1 Testicular Sperm Extraction (TESE)

Open TESE can be performed under intravenous sedation associated with locoregional anesthesia, local anesthesia only, or spinal block. It can be performed with or without testis delivered. The skin and subjacent layers are incised transversally to expose the tunica albuginea, which is opened with the knife. Usually, a small transversal albuginea opening (0.5–1.0-cm incision) is made at the mid-testicular pole, and a small sample of the parenchyma is cut off with scissors. The tunica is closed with a non-absorbable 5–0 running suture. The tunica vaginalis, dartos, and skin are sutured with absorbable suture [12, 15] (Fig. 50.3).

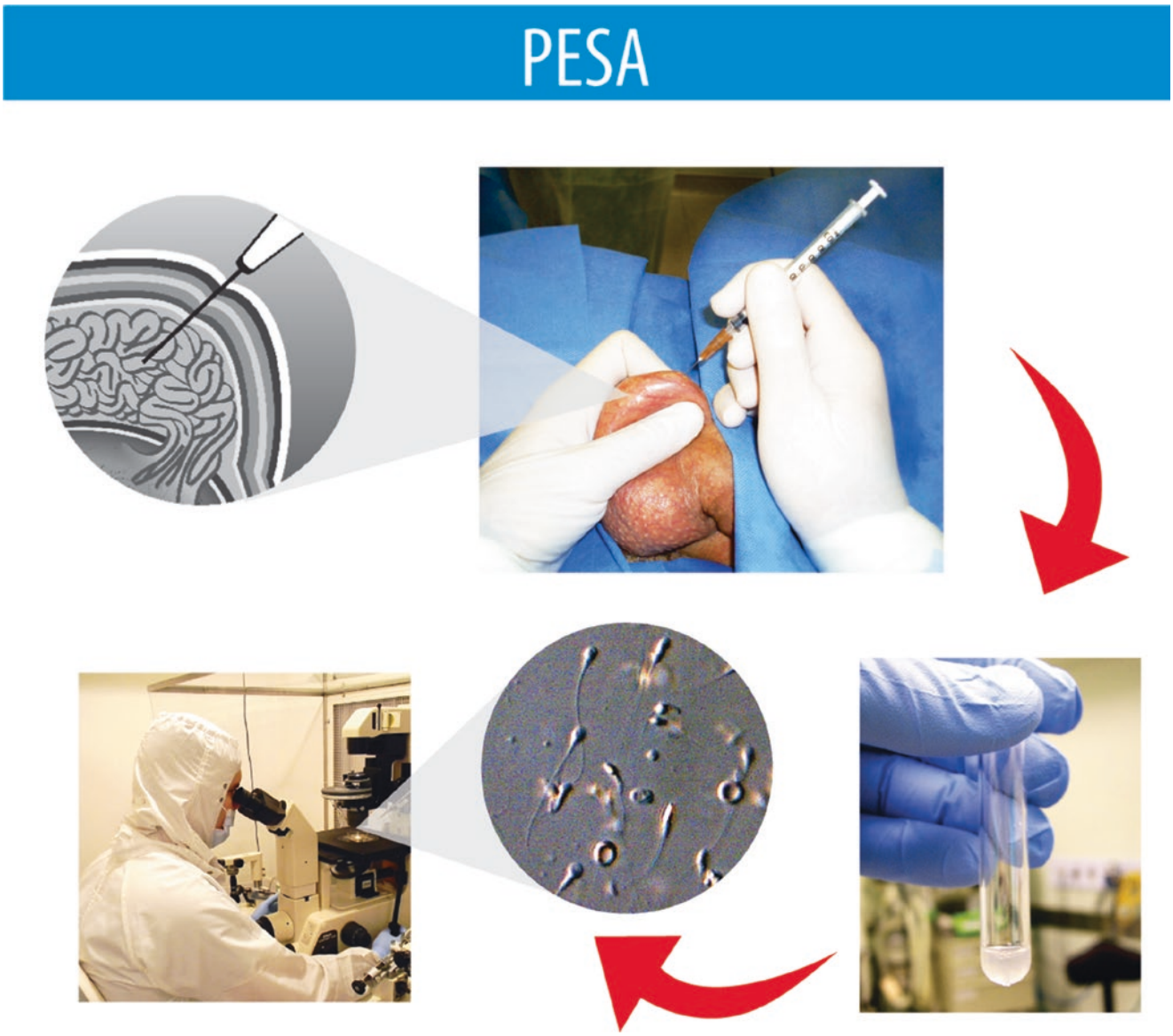

Fig. 50.3 Percutaneous epididymal sperm aspiration (PESA). Percutaneous aspiration of the epididymis is made using a thin needle connected to a syringe filled with sperm medium. Aspirate is sent for laboratory examination under the inverted microscope for sperm search (Reprinted with permission from ANDROFERT. All Rights Reserved)

50.2.3 Open Microsurgical

50.2.3.1 Epididymal Sperm Aspiration (MESA)

MESA is usually performed through a scrotal incision large enough to allow testis delivery. Since some degree of spermatic cord traction is expected unconsciousness is desirable for the procedure. General anesthesia under controlled ventilation may be used, but it is also possible to obtain sufficient analgesia with spermatic cord blockade using local anesthetics, associated with intravenous sedation with propofol.

An operating microscope and ×16–25 magnification are used to dissect the epididymal tunica and to open an opaque enlarged tubule with a microscopic knife or micro-scissors (Fig. 50.4). Culture media is added drop by drop over the

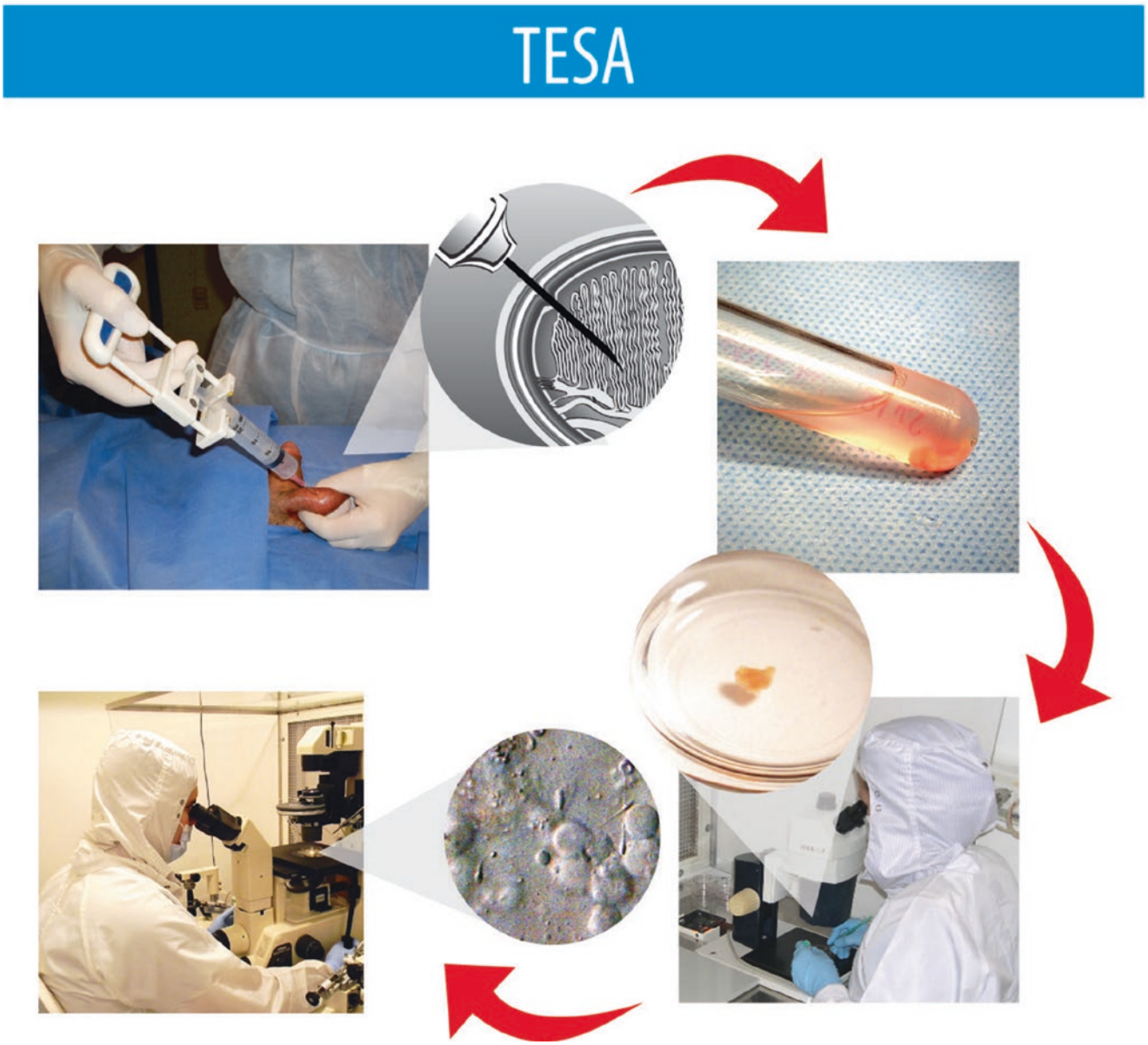

Fig. 50.4 Testicular sperm aspiration (TESA). Percutaneous aspiration of the testicle is carried out by inserting a 40 × 12-mm needle connected to a 20-mL syringe mounted on a syringe holder (e.g., Cameco syringe holder). The testis is firmly held, and the needle is moved in and out on various directions under negative pressure to disrupt and facilitate extraction of seminiferous tubules. Specimens are sent to the laboratory for mechanical mincing and examination under the inverted microscope for sperm search (Reprinted with permission from ANDROFERT. All Rights Reserved)

incised tubule to allow fluid aspiration and sperm recovery. If adequate sampling is not possible, another aspiration should be made more proximally than the first one toward the head of the epididymis in a different tubule. The proximal epididymal aspirate tends to have better quality than the distal ones; the latter is often more abundant in senescent sperm with reduced chromatin integrity [16]. If MESA fails to retrieve adequate numbers of motile sperm, TESA or TESE can be performed at the same side and operating time. However, MESA often permits the recovery of large numbers of high-quality sperm that can be used for immediate ICSI or cryopreserved for subsequent attempts avoiding additional surgical interventions [17].

50.2.3.2 Microdissection Testicular Sperm Extraction (Micro-TESE)

Micro-TESE is performed under intravenous sedation combined with local anesthesia or under general anesthesia or

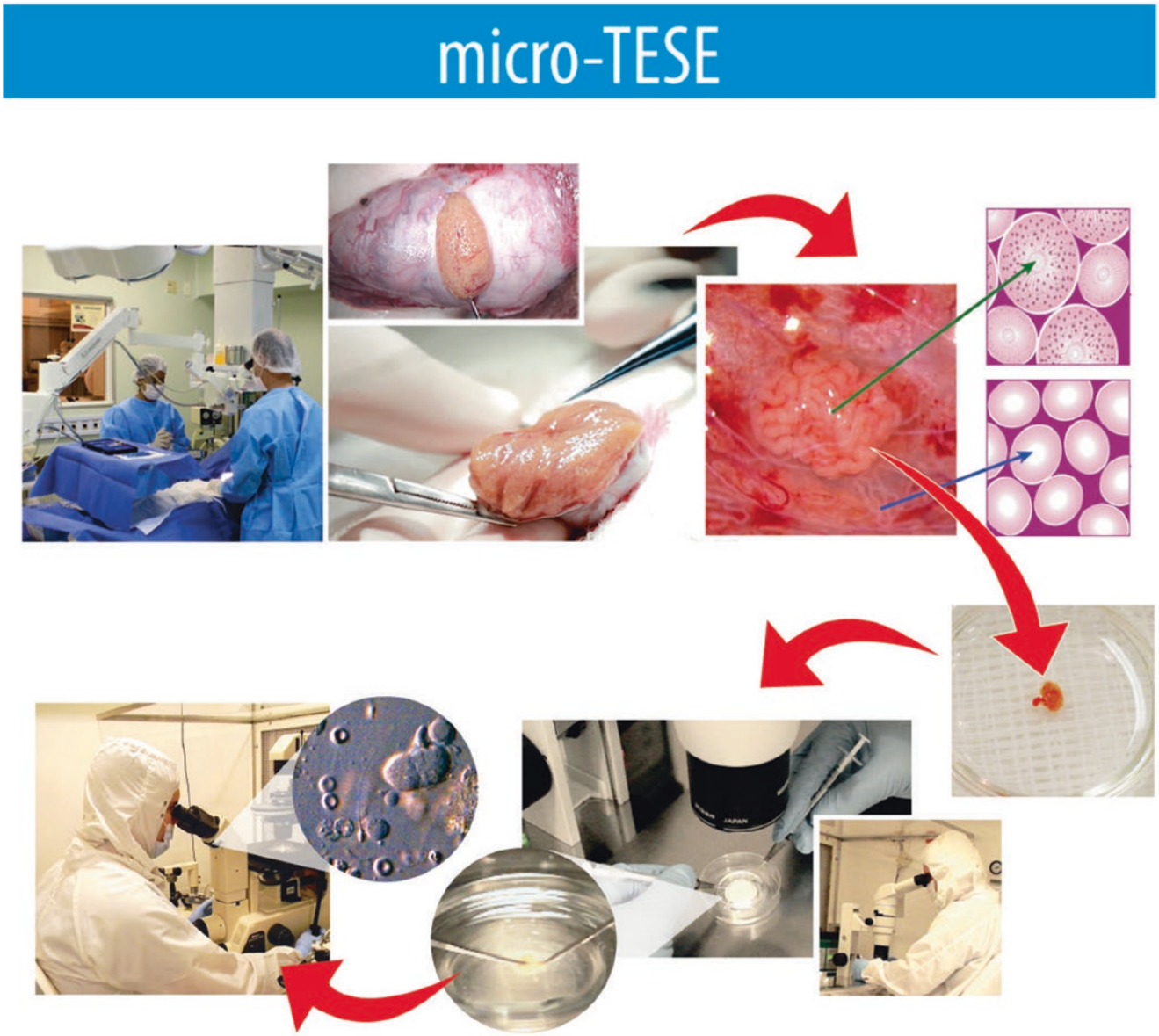

Fig. 50.5 Microdissection testicular sperm extraction (Micro-TESE). After testis delivery, a large equatorial incision is made in an avascular area of the tunica albuginea, and the testicular parenchyma is widely exposed. The seminiferous tubules are dissected at ×16 to ×25 magnification to enable identification of enlarged seminiferous tubules (see picture on the top right-hand side). Optical magnification reduces the chances of vascular injury by proper visual identification of testicular blood supply. Enlarged tubules are more likely to contain germ cells and therefore sperm production (green arrow shows the histological representation of an enlarged tubule exhibiting full spermatogenesis). Thin tubules are usually devoid of germ cells (blue arrow shows the histological representation of a thin tubule exhibiting Sertoli cells only). Microsurgical-guided biopsies are performed to extract enlarged tubules which are sent to the laboratory for examination. The initial laboratory step involves mechanical mincing of the seminiferous tubules and examination of specimens under the inverted microscope for sperm identification (Reprinted with permission from ANDROFERT. All Rights Reserved)

even spinal block [18]. After skin incision, the testis is delivered outside the scrotum. The tunica albuginea is then widely incised transversally, and the parenchyma is fully exposed (Fig. 50.5). Under microscope magnification of 16–25×, each testicular region is screened for enlarged seminiferous tubules as these have a higher chance to harbor germ cells and therefore sperm production [19]. If all tubules are identical, then random micro-biopsies (3–6 at each testicular pole) are recommended. Blood supply is actively avoided to preserve the remaining testicular parenchyma. Collected samples are immediately searched for sperm by the IVF lab. Given the initial sperm quantity and quality, the surgeon can

decide upon either ending the procedure or extending exploration to the contralateral side [12]. A short movie depicting the main steps of the procedure can be found at http://www.brazjurol.com.br/videos/may_june_2013/Esteves_440_441video.htm [20].

50.3 Prognostic Factors for Successful Sperm Retrieval

50.3.1 Obstructive Azoospermia

In patients with OA, spermatogenesis is preserved, and retrieval rates are virtually 100% regardless of the cause of obstruction [21]. However, sperm retrieved from the various parts of the seminal tract may vary in quality. The distal epididymis, for example, contains a high number of sperm fragments and macrophages [22]. The number of macrophages progressively decreases toward the proximal epididymis and testis, while the quantity of motile sperm gradually increases.

In one study, Esteves and colleagues evaluated sperm retrieval outcomes in 146 OA patients treated by ICSI [8]. The authors compared the results according to the cause of obstruction (congenital, vasectomy, or post-infection). In their study, epididymal sperm retrieval reached 78.0% of success, and testicular retrieval was able to rescue almost all failed epididymal attempts. Epididymal sperm retrieval was successful in all congenital cases, whereas in the other etiology groups (vasectomy, post-infectious obstruction), approximately 1/3 of patients required TESE. In the latter, the cumulative sperm retrieval rate (SRR) was 97.3% and did not differ among groups: CBAVD (100%), vasectomy (96.6%), and post-infection (96.3%) [8].

50.3.2 Nonobstructive Azoospermia

In NOA, few studies have looked into predictive factors for successful sperm retrieval. A recent systematic review showed that micro-TESE is more effective than conventional TESE as it offers higher SRR and lower surgical complications such as hematoma formation, fibrosis, and testicular atrophy [23]. Overall, SRR for conventional TESE and micro-TESE range from 17–45% and 25–63%, respectively. In the paper mentioned above, five out of seven included studies reported a significant difference regarding SR favoring micro-TESE ($p < 0.05$) [24–28].

Nonetheless, the main factor determining the odds of success in harvesting viable sperm with both techniques relates to testicular histology. Hypospermatogenesis (HS) and maturation arrest (MA) are associated with higher chances of harvesting sperm from the seminiferous tubules than Sertoli cell only (SCO) [8, 29, 30]. In a study involving 365 NOA patients undergoing micro-TESE, the SRR was higher in patients with MA (40.3%) than SCO (19.5%) ($P = 0.007$). Both groups did worse when compared with hypospermatogenesis (SRR = 100.0%; $p < 0.001$) [31]. Patients with SCO histopathology seem to benefit the most from micro-TESE. In SCO, success rates range from 22.5% to 41% using micro-TESE compared to 6.3% to 29% with conventional TESE [25, 28]. In patients with maturation arrest (MA), success rates are highly variable with some studies reporting SRR of 36.4% to 75% with the use of micro-TESE and 0% to 37.5% with conventional TESE [23]. In one study, however, there was a clear advantage of using micro-TESE in preference over conventional TESE in patients with MA [26]. The SRR with the former and latter approaches were 81–100% and 50–84%, respectively.

Other clinical predictors concerning SR success are serum follicle-stimulating hormone (FSH), inhibin levels, testicular volume, and testosterone levels. The studies by Okada et al. [25], Colpi et al. [27], and Ghalayini et al. [28] found FSH to be a predictive factor for successful sperm retrieval. Although no definitive cutoff value has been established, the authors reported that increased FSH levels were associated with significantly more failures in both TESE and micro-TESE [27, 28]. By contrast, Ramasamy et al. in a micro-TESE study involving men with NOA demonstrated that FSH levels have poor predictive value concerning success in sperm acquisition. In their study, patients with FSH levels >15 IU/mL had higher SRR than those with FSH levels <15 IU/mL [31]. In general, FSH levels correlate inversely with the spermatogenic status. However, despite reflecting the predominant testicular histology, FSH levels cannot be used to predict whether sperm-producing areas exist within the testis of a man with NOA. For instance, patients with failed SR might have normal FSH levels and normal-sized testes. This condition is explained by the presence of numerous Sertoli and germ cells (arrested at a specific spermatogenic stage); the former secrete adequate amount of inhibin that negatively feedback the FSH production [31].

Some studies suggest inhibin B to be more sensitive than FSH as an index of the spermatogenesis status [32, 33]. However, inhibin B, either alone or in combination with serum FSH, also fails to predict TESE outcomes in NOA patients and should not be used as a criterion to contraindicate the procedure [34]. Colpi et al. and Ghalayini et al. also evaluated testicular volume as a predictive factor for successful sperm retrieval but found that the data is equivocal [27, 28]. Along the same lines, a 2017 study reviewing the data of over 400 NOA patients found no significant difference in serum total testosterone levels in patients with successful and failed SR [35].

As for the location of the biopsy, Hauser et al. [36] could not demonstrate the advantage of performing the biopsy in any particular region of the testicle as a means to improve SR

success. However, Witt et al. suggested that the midline testicular area might provide the highest chance of harvesting sperm [37].

In one study including patients with Klinefelter syndrome (KS), the overall SRR was 51% (26/51) [38]. However, the authors could not find any predictive factor of success when analyzing FSH, LH, and testosterone levels, as well as testicular volume. By contrast, another study involving patients with non-mosaic KS found that advanced paternal age (>35 years old) adversely affected the SRR [39].

Testing for Y chromosome microdeletions is essential for counseling the affected men concerning the likelihood of SR success and risk of infertility in resulting male offspring [18, 40]. Among men diagnosed with complete AZFa and AZFb microdeletions, micro-TESE offers virtually zero chance of sperm recovery and therefore should not be encouraged [41].

Lastly, in the context of medication, Ramasamy et al. assessed the impact of preoperative hormonal therapy on SRR of KS patients. They concluded that patients who received hormonal therapy and responded with a resulting total testosterone level above 250 ng/dL (8.7 nmol/L) reached a SRR of 77% versus 55% in those who remained under this level [42]. In patients already receiving exogenous testosterone replacement, the pituitary is suppressed, and therefore cessation is recommended for at least 6 months before microdissection to allow gonadal axis reestablishment [43].

Enhancing intratesticular testosterone production and correcting abnormalities in the ratio of testosterone to estrogen have been advocated to optimize SR success. In a multicenter nonrandomized study involving 442 subjects diagnosed with NOA, SR was higher in the hormonal optimization group than in the group that underwent SR without previous hormonal treatment (57% versus 34%; $p < 0.05$) [44]. Nevertheless, the quality of evidence currently available is very low to recommend routine hormonal optimization therapy [18, 39].

50.4 Complications

Postoperative complications following SR include persistent pain, swelling, infection, hydrocele, and hematoma [26, 45, 46]. Ultrasound scans performed 3 months after single or multiple biopsy TESE reveal the presence of intratesticular hematoma in approximately 80% of patients, which tend to resolve spontaneously without compromising testicular function [46, 47].

In large-volume conventional TESE, however, temporary or definitive testicular damage (such as complete devascularization) might decrease serum T levels [26, 48]. TESA and micro-TESE minimize the risk of complications and long-term adverse consequences, including hypogonadism [7, 18, 26, 46, 48, 49].

In micro-TESE, subalbuginea vessels are spared during the testicular opening [20]. The use of optical magnification and microsurgical technique not only allow preservation of intratesticular blood supply but also increase the chances of identifying sperm-producing tubules [18, 20, 26]. Hence, SR efficacy is optimized since the risk of complications, and the quantity of tissue removed is reduced. The smaller amount of tissue extracted – compared to conventional TESE – speeds up tissue processing and sperm search [20, 50]. In a large cohort study involving 435 NOA patients subjected to micro-TESE or conventional TESE, postoperative ultrasound examination confirmed that micro-TESE caused fewer acute and chronic testicular changes than TESE [26]. The authors of the study mentioned above reported that although there is an initial reduction in T levels after micro-TESE, such levels return to 95% of their preoperative values in an 18-month follow-up period. These findings have been corroborated by others [51].

Nevertheless, men with severely hypotrophic testes and low serum T levels (e.g., Klinefelter syndrome) might have a more significant reduction in T levels, thus being at a higher risk of requiring permanent T replacement therapy [52]. In one report involving KS men, serum T levels significantly declined by 30–35% ($p < 0.01$) after micro-TESE over a 1- to 12- month period, but returned to 75% of the preoperative levels after 18 months [43]. Given the potential risk for severe adverse effects, it is critical that sperm retrieval in NOA men be performed by well-trained surgeons [48].

50.5 Assisted Reproductive Technology

50.5.1 Role of IVF Laboratory

In general, sperm processing techniques are needed to remove cellular debris, microorganisms, and red blood cells that might contaminate the extracted specimens. These methods should be mastered to avoid deteriorating the sperm fertilizing potential further since the quality of surgically retrieved sperm is often lower than ejaculated counterparts [52]. Processed sperm can be either used for immediate ICSI or cryopreserved for future use.

From the surgeon's perspective, all efforts should be made to deliver specimens with minimal or no contaminants to the IVF laboratory. Lab personnel, on its turn, should minimize iatrogenic cellular damage during sperm preparation. Controlling centrifugation force and duration, limiting exposure to ultraviolet light and temperature variation, optimizing laboratory air quality conditions, and using high-quality reagents, culture media, and disposable materials are critical elements [53]. Whenever possible, techniques aimed at improving the sperm fertilizing potential should be applied, including the use of chemical stimulants and/or methods to

Table 50.1 Laboratory strategies to handle surgically extracted sperm

Process	Procedures	Techniques	Main goal
Testicular tissue processing	Micro-TESE	Extraction of minimum volume of tissue	Speed up tissue processing and search efficiency in men with NOA
Testicular tissue processing	Mechanical tissue mincing	Disruption of seminiferous tubules using fine needles or micro-scissors and forced pass through small-diameter catheters	Tubular breakdown and cellular content loss
Testicular tissue processing	Enzymatic tissue digestion	Incubation of testicular suspensions with collagenase and/or DNAse	Tubular breakdown and cellular content loss in men with NOA
Testicular tissue or epididymal fluid processing	Red blood cell lysis	Incubation of testicular or epididymal suspensions with erythrocyte lysing buffer solution	Removal of excessive blood cells from testicular or epididymal specimens
Testicular tissue of epididymal fluid processing	Motility enhancement	Incubation of testicular specimens of epididymal fluid with pentoxifylline	Selection of viable sperm for ICSI
Laboratory environment and laboratory practices	Air quality control	Air particulate and volatile organic compound filtration	Secure optimal safety conditions for gamete handling, sperm injection, and embryo culture
	Maintenance of temperature and pH stability	Quality control and quality assurance of instruments, equipment, and reagents	Avoid iatrogenic cellular damage
	Centrifugation	Simple washing with buffered medium or mini-gradient centrifugation using low centrifugation forces	Avoid iatrogenic cellular damage
	Sterile techniques	Manipulation of gametes and embryos in laminar flow cabinets or inside controlled environments	Secure optimal safety conditions for gamete handling, sperm injection, and embryo culture
Intracytoplasmic sperm injection	Sperm selection	Hypoosmotic swelling test, mechanical touch technique, and laser-assisted sperm selection	Selection of viable immotile sperm for ICSI
Sperm storage	Cryopreservation	Sperm freezing using low-volume carriers	Improve post-thaw sperm survival

ICSI intracytoplasmic sperm injection, *NOA* nonobstructive azoospermia

select viable sperm for ICSI. The latter is particularly important when only immotile spermatozoa are harvested [54]. An overview of the laboratory aspects concerning the processing of surgically extracted specimens is provided in Table 50.1. A detailed laboratory procedure for processing such specimens can be found elsewhere [12, 17].

50.5.2 Influence of Type of Azoospermia

Although spermatogenesis is normal in cases of OA, ICSI rather than conventional IVF should be the fertilization method to be used with sperm retrieved from both the epididymis and testicle due to the low fertilizing capacity of such gametes in conventional IVF [54, 55]. With ICSI, the use of fresh or frozen-thawed sperm harvested from the epididymis or seminiferous tubules from men with OA does not seem to affect outcomes [56, 57]. However, the evidence is not categorical as a retrospective study involving 374 men with OA reported that the likelihood of achieving a live birth was higher with epididymal than with testicular sperm (OR 1.82, 95% CI 1.05–3.67) [12].

A meta-analysis pooling 100 ICSI cycles compared ART outcomes in OA according to congenital or acquired causes [58]. Men with CBAVD achieved higher fertilization rates than those with acquired obstruction (p = 0.04). In their study, no difference was noted in clinical pregnancy rates (CPR) and LBR between groups, but miscarriage rates were higher in the congenital group (RR ~ 2.7). By contrast, Kamal et al. studied 1661 ICSI cycles in 1121 men with proven histological OA (normal spermatogenesis). Mean female partner age was 30.9 ± 5.7 years (17–45 years). Implantation rate (IR) (19.9% vs. 20.8%), CPR (43.2% vs. 42.3%), and miscarriage rate (18.4% vs. 17.6%) were not significantly different when testicular or epididymal sperm were used for ICSI, respectively. The same trend was noted concerning the cause of obstruction (CBAVD vs. acquired obstruction), thus suggesting that ICSI success is independent of the factors discussed above [59]. The 2PN fertilization rate (68.0% vs. 64.2%, p = 0.02) was the only significant parameter favoring testicular sperm.

In another study, Esteves et al. retrospectively analyzed 146 men with OA to compare ICSI results according to the cause of obstruction (congenital versus acquired). Live birth rates (LBRs) were similar among congenital (34.4%), vasectomy (32.2%), and post-infection groups (36.4%). Clinical pregnancy rates, miscarriage rates, and prematurity and low birth weight rates were also not significantly different [10].

Sukcharoen et al. studied the influence of time of obstruction on ICSI outcome. They reported on a cohort of 17

patients and 21 ICSI cycles within a period of 2 years, analyzing 3 groups according to the time elapsed since vasectomy: 0–10 years, 11–20 years, and more than 20 years. Fertilization rate, IR, and CPR per transfer were not significantly different among the groups [60]. However, this cohort was too small to allow any conclusion.

ICSI outcomes seem to favor OA over NOA, which is not surprising since spermatogenesis is considered normal in the former. In OA, the method of sperm retrieval – percutaneous or open surgery – and the site of sperm acquisition, testis or epididymis, may be chosen according to the preference and expertise of the attending urologist. There is no solid evidence that the site or method of sperm retrieval influences the outcome of ICSI for patients with OA [61, 62]. Additionally, neither the cause of obstruction nor the use of fresh or frozen-thawed epididymal/testicular sperm seems to have any significant effect on the success of ICSI regarding fertilization, pregnancy, or miscarriage rates. ICSI provides fertilization rates of 45–75% per injected oocyte when epididymal or testicular spermatozoa from men with OA are used. In such cases, CPR and LBR range from 26 to 57% and 18 to 55%, respectively [21, 59, 63–66].

By contrast, the reproductive outcomes of men with NOA subjected to ICSI with testicular sperm harvested from the seminiferous tubules are less optimal. In one study, Esteves and Agarwal compared sperm injection outcomes using fresh surgically extracted sperm from men with OA (182 cycles) and NOA (188 cycles) to those from a general population of infertile men using freshly ejaculated sperm for injections (621 cycles) [67]. The lowest LBRs were reported in men with NOA (21.4%; p = 0.003), whereas men with OA (37.5%) and those from the general male infertility population using ejaculated sperm (32.3%) had similar LBRs. In this study, ICSI outcomes were comparable between obstructive azoospermia and ejaculated sperm groups. In another report, ICSI outcomes were compared in NOA men with successful (n = 365) and failed (n = 40) SR by micro-TESE [29]. ICSI was carried out with testicular sperm and donor sperm, respectively. Live birth rates in both groups were compared with those from a group of 186 men with OA in whom epididymal or testicular sperm were used for ICSI. The adjusted OR showed that the likelihood of achieving a live birth was lower in men with NOA who had successful SR than in those with NOA in whom donor sperm was used (OR 0.377, 95% CI 0.233–0.609; p < 0.001) and to men with OA (OR 0.403, 95% CI 0.241–0.676; p = 0.001) [29]. The authors also noted that fertilization rates after ICSI (47% vs. 61–64%, p < 0.01), high-quality embryo rates (43% vs. 61–66%, p < 0.01), and CPR (28% vs. 47–50%, p < 0.01) were lower in NOA men with successful SR than in both men with NOA in whom donor sperm was used and OA [29].

50.5.3 Sperm Retrieval in Non-azoospermic Men

Sperm retrieval from epididymides or seminiferous tubules for use with ICSI is the clear strategy for overcoming untreatable azoospermia-related infertility [68]. Recently, testicular sperm retrieval has also been used in non-azoospermic men to bypass post-testicular oxidative-induced DNA fragmentation.

Indeed, current data indicate that among non-azoospermic infertile men, sperm harvested from the seminiferous tubules have threefold to fivefold lower sperm DNA fragmentation (SDF) – a marker of chromatin quality – than ejaculated sperm [69–72]. Given the importance of sperm chromatin integrity for ART success, the use of testicular in preference over ejaculated sperm for ICSI has gained increasing attention. The aim is to increase the chances of oocyte fertilization by genomically intact sperm, which might ultimately result in the development of embryos with higher implantation potential.

In one prospective cohort study, Esteves et al. compared ICSI results with the use of ejaculated and testicular sperm in a population of 172 infertile men with high SDF [70]. The authors included infertile men with mild-to-moderate idiopathic oligozoospermia (5–15 × 10^6 spermatozoa per mL) who presented with persistent high SDF (>30%) even after using oral antioxidant therapy for at least 3 months. SDF was re-assessed in both ejaculated and testicular specimens on the day of sperm collection for ICSI using the sperm chromatin dispersion test (SCD). Paired ejaculated and testicular specimens from the same men showed that SDF rates were fivefold higher (40.7% ± 9.9%) in the ejaculate than in the testis (8.3% ± 5.3%; P < 0.001). In this group, ICSI was performed with testicular sperm (Testi-ICSI). In patients subjected to ICSI with ejaculated sperm, SDF rates were 40.9% ± 10.2%. Miscarriage rates were lower, and LBRs were higher in couples treated with testicular sperm than with ejaculated sperm. The adjusted relative risks for miscarriage and live birth between testicular sperm and ejaculated sperm groups were 0.29 (95% CI 0.10–0.82; p = 0.019) and 1.76 (95% CI 1.15–2.70; p = 0.008), respectively. The authors reported that five couples needed to be treated (NNT; 95% CI 2.8–16.8) by testicular compared to ejaculated sperm to obtain an additional live birth per fresh transfer cycles [70]. These data indicate that using Testi-ICSI, it would be possible to avoid one out of five oocyte pickups [73].

In another study, Bradley et al. also compared ICSI outcomes between ejaculated and testicular sperm in non-azoospermic men with high SDF in the ejaculated sperm [74]. Among patients in the ejaculated sperm group, the authors applied interventions such as IMSI (intracytoplasmic morphologically selected sperm injection) and HA sperm selection ICSI (physiological ICSI (PICSI)) to select sperm

with less DNA fragmentation and compared outcomes with a control group in which no particular intervention was used to select sperm with an intact chromatin. The authors evaluated the results of ICSI using ejaculated sperm – with (228 cycles) and without such interventions (80 cycles) – or testicular sperm (Testi-ICSI; 148 cycles). Higher LBRs ($p < 0.05$) were obtained with Testi-ICSI (49.8%) than IMSI (28.7%) and PICSI (38.3%). The lowest LBRs (24.2%) were achieved when no intervention was used to avoid the use of sperm with fragmented DNA ($p = 0.020$) [74].

A 2017 systematic review followed by meta-analysis corroborated the findings of the studies mentioned above concerning (i) the lower rates of SDF in testicular sperm than in ejaculated sperm and (ii) the better ART outcomes with the use of testicular in preference over ejaculated sperm for ICSI in men with high SDF in the semen [11]. By contrast, the benefit of using testicular sperm rather than ejaculated sperm for ICSI has not been confirmed among men with cryptozoospermia. A meta-analysis pooling five small case-control and observational studies with a total of 300 cycles looked at ICSI results in this scenario [75]. The authors reported no differences in fertilization rates (RR 0.91, 95% CI 0.78–1.06) and pregnancy rates (RR 0.53; 95% CI 0.19–1.42) when testicular sperm was compared with ejaculated sperm.

Currently, the advantage of using Testi-ICSI over ejaculated sperm ICSI in non-azoospermic men seems to be restricted to men with confirmed abnormally high SDF in the semen; in these cases, a favorable outcome with testicular sperm has been found regarding clinical pregnancy, miscarriage, and live birth [69, 70, 73, 74, 76–79].

50.6 Health of Offspring

The widespread use of surgically retrieved sperm for ICSI has raised concerns about the health of resulting offspring owing to the related severe male infertility conditions and because such gametes have not completed full maturation. Concerns include possible increased risk for congenital and urogenital malformations, epigenetic alterations, chromosomal aneuploidies, infertility, childhood cancer, delayed psychological and neurological development, and impaired cardiometabolic profile.

In general, any increase in these conditions is believed to be consequential of parental sperm defects rather than the ART method [68]. In fact, the integrity of the sperm genome and epigenome is essential to assure healthy offspring [80]. Several environmental insults can damage histone-bound sperm DNA including oxidative stress. The male gamete is highly vulnerable to free radical-induced DNA damage since the majority of cytosolic antioxidants during spermiogenesis are lost. Persistent DNA damage in ejaculated sperm from subfertile men exposed to in vitro conditions can be partially explained by low levels of essential DNA repair enzymes [81, 82]. When used for ICSI, DNA-damaged sperm may lead to an increased risk for fertilization failure, poor embryo development, abortion, congenital malformations, childhood cancers, and perinatal morbidity [80, 83].

Current evidence suggests that children born through ICSI have an increased risk of congenital malformations and chromosomal abnormalities (~1.0%) when compared with naturally conceived children (~0.2%) or conventional IVF (~0.7%) [68, 84–88]. Additionally, childhood cancer and disrupted reproductive hormonal profile have been observed in offspring born from ICSI compared with naturally conceived counterparts [68]. Lastly, epigenetic disorders and impaired neurodevelopment have also been observed in infants born from ICSI compared with naturally conceived children. The underlying parental infertility seems to have a significant effect on the health of ICSI offspring [68].

Whether the risk of health issues is increased further in infants born through ICSI using surgically retrieved sperm is unknown. The literature is scanty on this matter, but the existing data from ICSI studies evaluating congenital and chromosomal abnormalities in offspring of azoospermic fathers who have used epididymal or testicular sperm for ICSI are reassuring overall [67]. The current studies indicate that congenital malformation rates (~1.6%) and short-term neonatal outcomes are comparable between infants born through ICSI from OA and NOA fathers [67, 89, 90]. Additionally, these rates seem not to differ when the overall population of children born from azoospermic fathers is compared to that born from non-azoospermic fathers subjected to ICSI with ejaculated sperm [91, 92]. Some evidence does, however, suggest that autistic disorder and mental retardation might increase in children born after ICSI and TESE to treat azoospermia compared to conventional IVF. In a prospective cohort study involving 30,959 children born after ART and 2,541,155 children conceived naturally, autistic disorder (adjusted RR 4.60, 95% CI 2.14–9.88) and mental retardation (adjusted RR 2.35, 95% CI 1.01–5.45) were higher after ICSI using surgically extracted sperm than in IVF, although the association was not evident among singletons (RR 0.70, 95% CI 0.10–5.16) [93]. As the published data lack a strong level of evidence, these associations demand further investigation.

50.7 Conclusion

Sperm retrieval techniques are widely used to harvest sperm from the epididymis or seminiferous tubules, in particular, in men with azoospermia. Surgically retrieved gametes are used for intracytoplasmic sperm injection (ICSI). In men with obstructive azoospermia, both percutaneous and open sperm retrieval methods are highly effective to retrieve sperm

from the epididymis or testes. In nonobstructive azoospermia, open testicular sperm retrieval is the method of choice, preferably using a microsurgical approach. Lately, testicular sperm retrieval has been used successfully to retrieve sperm with better chromatin integrity from non-azoospermic men. Overall, sperm retrieval methods have low complication rates. ICSI outcomes mainly depend on the type of azoospermia rather than the method used to harvest sperm, with less favorable results in men with nonobstructive azoospermia. The health of offspring from ICSI using surgically retrieved gametes is overall reassuring. However, a call for continuing monitoring is warranted as the underlying parental infertility might increase the risk of congenital malformations, epigenetic disorders, chromosomal abnormalities, subfertility, cancer, delayed psychological and neurological development, and impaired cardiometabolic profile. It remains to be determined to what extent the observed adverse outcomes might be aggravated by using surgically retrieved sperm.

50.8 Review Criteria

A search of studies examining the use of surgical techniques to retrieve sperm from the testes and epididymides from infertile men for intracytoplasmic sperm injection was performed using PubMed and MEDLINE. The start date for the search was not specified, and the end date was November 2018. The overall strategy for study identification and data extraction was based on the following keywords: "male infertility"; "sperm retrieval"; "epididymal sperm"; "testicular sperm"; "azoospermia"; "reproductive techniques, assisted"; "ICSI"; "in vitro fertilization"; "sperm injections, intracytoplasmic"; and "IVF," with the filters "humans" and "English language." Our search did not include the use of surgical and non-surgical sperm retrieval techniques in patients with ejaculatory dysfunctions as the matter concerned was out of the scope of this chapter. Citations from book chapters and grey literature were only included if provided with conceptual contents.

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