Sperm retrieval techniques

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Sperm retrieval techniques

Daniel H. Shin and Paul J. Turek

Abstract | Since the advent of intracytoplasmic sperm injection in 1992, sperm retrieval procedures have been routinely employed to treat male infertility owing to azoospermia. With obstructive azoospermia, sperm is potentially harvestable from the vas deferens, epididymis, and testicle using percutaneous and open sperm retrieval procedures that are relatively straightforward and reliable. In nonobstructive azoospermia, sperm is generally found only in the testicles and can often be difficult to retrieve. Several approaches aimed at maximizing sperm yield in this condition have been developed, but only 50% of men with nonobstructive azoospermia will have clinically usable sperm. Multibiopsy testicular sperm extraction (TESE), microdissection TESE, and fine-needle-aspiration map-guided TESE are three common methods currently employed to locate and retrieve sperm in these difficult cases. Other factors that influence the use of surgically retrieved sperm for assisted reproduction include differences in sperm DNA integrity, the expertise of the surgeon and the andrology laboratory, and the described differences in the viability of sperm from different anatomical sources after freezing and thawing.

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Introduction

Azoospermia is the absence of spermatozoa in the semen on two centrifuged semen samples. It affects 1% of the general population and up to 15% of infertile men.[1] Since the advent of *in vitro* fertilization (IVF) in 1978 and intracytoplasmic sperm injection (ICSI) in 1992,[2] men with azoospermia have had the opportunity to conceive. Retrieving sperm from the male reproductive tract, first described in 1988, is now routine with ICSI, and with robust success.[3]

Azoospermia is divided into either obstructive or nonobstructive types based on aetiology. Obstructive azoospermia is caused by congenital or acquired conditions that obstruct the passage of sperm from the testicle through the reproductive tract. These conditions are varied and include congenital absence of the vas deferens, prior vasectomy, infections, ejaculatory duct obstruction, and idiopathic causes. Sperm retrieval with reproductive tract obstruction can therefore be tailored to the condition and target the vas deferens, the epididymis, or the testicle for sperm (Figure 1).

Nonobstructive azoospermia is a consequence of testicular failure, with an associated lower than normal production of mature sperm. Common primary causes include Y chromosome microdeletions or karyotype abnormalities, chemotherapy, infection, cryptorchidism, or torsion. Sperm retrieval in these conditions, if possible, is limited to the testicle. Secondary causes, including Kallmann syndrome and prolactinoma, are due to faulty hypothalamic and pituitary signalling and are often hormonally correctable.

Competing interests
The authors declare no competing interests.

Some important points deserve to be mentioned before sperm retrieval techniques are reviewed. A Cochrane meta-analysis suggested that evidence is currently insufficient to recommend one specific sperm retrieval technique over another for either obstructive or nonobstructive azoospermia.[4] This uncertainty has opened the door to individually tailored sperm retrieval procedures to optimize success and safety. This individualized approach is especially critical in cases of nonobstructive azoospermia, in which sperm retrieval can be complex and often fails. Also, as many couples might require multiple ICSI cycles for successful conception and birth, the onus is on clinicians to develop efficient sperm retrieval techniques that maximize yield and minimize procedure numbers and morbidity.[5]

Using principles of evidence-based medicine, this Review incorporates findings from randomized controlled trials, basic scientific studies, meta-analyses, case-controlled or cohort studies, best-practice policy recommendations, and literature reviews to discuss the current understanding of sperm retrieval techniques and strategies. In addition, we appraise the health of sperm from various reproductive tract sources and clarify the value of sperm cryopreservation in reproductive medicine.

Vasal sperm aspiration

Men with azoospermia due to congenital or acquired obstruction at the level of the prostate or along the vas deferens are candidates for vasal sperm aspiration. Acquired obstruction is classically a sequela of infection or surgery (for instance, from vasectomy, after radical prostatectomy, or following mesh inguinal hernia repair). Ejaculatory failure secondary to reproductive

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Key points

- All men with obstructive azooospermia and many men with nonobstructive azoospermia will have sperm for biological pregnancies
- Evidence is currently insufficient to recommend any specific sperm retrieval technique over another for either obstructive or nonobstructive azoospermia
- The onus is on clinicians to develop efficient sperm retrieval techniques that maximize yield and minimize procedure numbers and morbidity
- Obstructive azoospermia cases are associated with higher normal fertilization rates and clinical pregnancy rates compared with nonobstructive azoospermia cases
- When fresh and frozen–thawed testicular sperm are compared, fertilization, clinical pregnancy, and ongoing clinical pregnancy rates are not different, but there is a significant decrease in implantation rates with frozen–thawed sperm

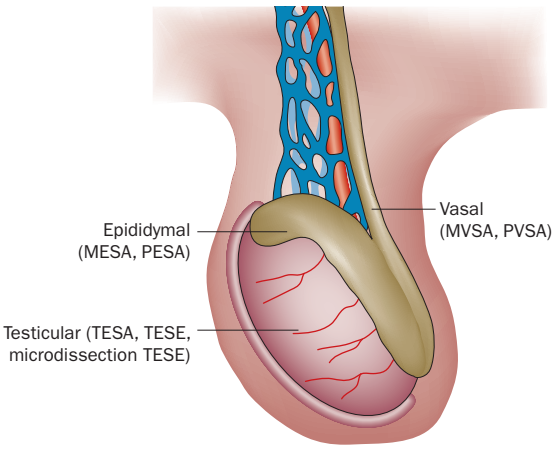

Figure 1 | Sites of sperm retrieval. Sperm retrieval is theoretically possible and commonly performed in three reproductive tract organs: the vas deferens, epididymis, and testicle. Abbreviations: MESA, microscopic epididymal sperm aspiration; MVSA, microscopic vasal sperm aspiration; PESA, percutaneous epididymal sperm aspiration; PVSA, percutaneous vasal sperm aspiration; TESA, testicular sperm aspiration; TESE, testicular sperm extraction. With kind permission from Springer Science+Business Media B. V. © Turek, P. J. in *Reproductive Endocrinology and Infertility: Integrating Modern Clinical and Laboratory Practice.* (eds Carrell, D. T. & Peterson, C. M.) 453–465 (Springer, 2010).

tract dysfunction without physical blockage, such as that observed in patients with spinal cord injury, multiple sclerosis, or diabetes mellitus, can also be managed with vasal aspiration. Alternatively, in cases of dysfunctional ejaculation resulting from spinal cord injury, rectal probe electroejaculation and penile vibratory stimulation are reliable and noninvasive ways of retrieving high quality ejaculated sperm.[6]

Patients with suspected obstructive azoospermia are evaluated by history and by physical examination, with attention to determining the presence or absence of the scrotal vas deferens and epididymides. The evaluation should reveal normal testicular volume, and normal testosterone and follicle stimulating hormone (FSH) levels. However, before assuming obstruction in all such cases, one must be aware that a particular form of non-obstructive azoospermia, which is due to early maturation arrest, can closely mimic the clinical features of obstruction but has no mature sperm production.[7]

Vasal aspiration is performed in the same manner as classic vasography, and can generally be done under local anaesthesia or using mild intravenous sedation in a no-scalpel manner. With microscopic vasal sperm aspiration, the vas deferens is secured through a scrotal puncture. With the assistance of a loupe or microscopic magnification, a small puncture or hemi-incision is made in the muscular wall of the vas deferens (Figure 2). Next, a fine-tipped (24 gauge) syringe is used to aspirate the sperm-rich fluid from the vas deferens lumen until 2–20 million motile sperm are collected. Applying pressure or massaging the testis vas deferens can help express fluid for aspiration. In cases of hemi-incision, the vas deferens wall is closed with microscopic sutures. Recovery is expected within 24 h, with minimal adverse effects including bleeding (<1%) and infection (<1%). There is a small potential (<5%) for stenosis of the vas deferens lumen due to cicatrix formation. With the percutaneous approach, a small (30 gauge) needle is inserted into the vasal lumen through which fluid and sperm are aspirated without incising the vas deferens. The main advantage of vasal sperm aspiration lies in the maturity of the sperm retrieved. Vasal sperm have undergone the full maturation sequence associated with epididymal passage. As such, this sperm can be used not only for ICSI, but also for intrauterine insemination and IVF, if suitable numbers are obtained (Table 1).[8–10] In addition, vasal sperm is well-suited for cryopreservation as it demonstrates a high level of post-thaw viability and superior motility compared with testicular sperm (Table 2).[11] On the basis of the generally high biological quality of vasal sperm, aspiration can be timed to occur on the same day as partner ovulation or frozen in advance of ovulation and thawed at the time of oocyte retrieval.

Epididymal sperm aspiration

Epididymal sperm aspiration is indicated for patients in whom the vas deferens is either absent or obstructed, as observed with congenital absence of the vas deferens or as sequelae of infection, trauma, or prior surgery. Similar to vasal sperm aspiration, epididymal sperm aspiration can be performed on the same day as partner ovulation, or retrieved sperm can be frozen in advance and thawed for egg retrieval.

Two surgical approaches to epididymal sperm aspiration are popular: microscopic epididymal sperm aspiration (MESA) and percutaneous epididymal sperm aspiration (PESA). Both are performed under local anaesthesia, with or without light sedation. With MESA, a small (<1 cm) incision is made in the superior hemiscrotum overlying the caput epididymis (Figure 3).[12] A surgical microscope aids identification of dilated epididymal tubules, which are then individually incised or punctured with micro-instruments. Epididymal fluid is aspirated until 2–10 million motile sperm are collected, and the overlying tunic is closed with microscopic sutures. Recovery and complications are similar to those for vasal sperm aspiration except that there is no concern about vasal stenosis.[12]

During PESA procedures,[13] the testicle is isolated and the scrotal skin stretched taut over its upper pole. The caput epididymis is palpated and then percutaneously punctured with a small-gauge butterfly needle until sufficient motile sperm are collected. If no sperm-containing fluid is obtained, several redirected needle passes are performed throughout the caput epididymis. Pressure is applied for several minutes for haemostasis.

The major differences between the MESA and PESA procedures are expense and reliability. Operative microscopy use in MESA increases procedural costs relative to PESA. However, not all epididymal tubules contain sperm of similar motility, and by enabling sampling of individual epididymal tubules, MESA allows the selection of sperm with maximum motility. With PESA, several epididymal tubules are sampled blindly, resulting in an aspirated specimen with an average motility that is generally lower than with MESA. MESA procedures are more likely to reduce the need for repeat sperm retrievals compared with PESA procedures. Patient recovery is similar between the procedures.

Epididymal sperm exhibit variable levels of maturation and generally less motility than vasal sperm.[11] Although pregnancies have been reported with IVF, epididymal sperm is best suited for ICSI, with good oocyte fertilization (56–64%) and pregnancy (32–50%) rates reported.[5,14–16]

Testicular sperm retrieval

The successful use of ICSI with testicular sperm was first reported by Craft *et al.*[17] in 1993, and was a great leap forward for the field, as it demonstrated that sperm do not require epididymal maturation to be able to fertilize an oocyte. Testicular sperm extraction is now used routinely in obstructive azoospermia and is essential for men with testicular failure, or nonobstructive azoospermia.

Obstructive azoospermia

Men with obstructive azoospermia at the level of the efferent ducts or beyond, or men who are functionally obstructed owing to neurological conditions are candidates for testis sperm retrieval. In these patients, testicular sperm can be retrieved by needle aspiration (testicular sperm aspiration; TESA), by percutaneous biopsy or by open surgical biopsy (testicular sperm extraction; TESE).

As with the other aspiration procedures, TESA is performed on an outpatient basis with or without intravenous sedation. It can be timed to occur with partner oocyte harvest, or it can be performed in advance with cryopreservation. The technique involves stabilization of the testicle in the scrotal sac between the thumb, index, and middle fingers. Next, a butterfly needle or an angiocatheter needle is directed into the testicular parenchyma through the scrotal skin. If an angiocatheter needle is used, the hollow needle is withdrawn, leaving the soft plastic sheath within the testicle. Negative pressure is applied through a syringe connected with tubing to the needle or catheter and the needle is passed back and forth within the substance of the testicle while maintaining the vacuum seal. When sufficient tissue is collected, the suction is released and the needle is removed from the testicle. Using air or wash medium, the aspirated tissue is flushed into a Petri dish or collection tube. After fine dissection with scissors or scalpels, the tissue-fluid is examined with microscopy to confirm sperm presence. Repeat sperm aspiration can be performed on the same

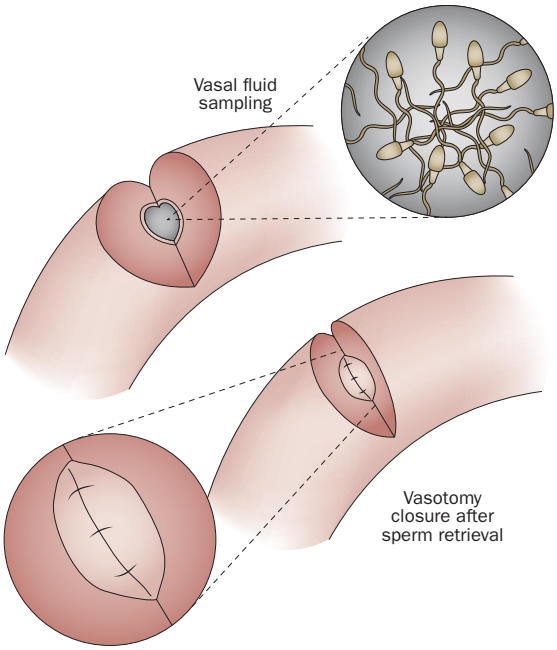

Figure 2 | The vasotomy approach to sperm aspiration. After no-scalpel delivery, a hemi-vasotomy is made in the vas deferens under magnification. After sperm is retrieved, the vasal incision is closed in two layers with microscopic sutures to minimize stenosis.

Table 1 | Utility of retrieved sperm in ART

Sperm retrieval procedure	IUI	IVF	ICSI
Vasal aspiration (MVSA, PVSA)	Maybe	Yes	Yes
Epididymal aspiration (MESA, PESA)	No	Yes	Yes
Testicular retrieval (TESA, TESE, microdissection TESE)	No	Yes	Yes

Abbreviations: ART, assisted reproductive techniques; ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; IVF, *in vitro* fertilization; MESA, microscopic epididymal sperm aspiration; MVSA, microscopic vasal sperm aspiration; PESA, percutaneous epididymal sperm aspiration; PVSA, percutaneous vasal sperm aspiration; TESA, testicular sperm aspiration; TESE, testicular sperm extraction. With kind permission of Springer Science+Business Media B. V. © Turek, P. J. in *Reproductive Endocrinology and Infertility: Integrating Modern Clinical and Laboratory Practice*. (eds Carrell, D. T. & Peterson, C. M.) 453–465 (Springer, 2010).

Table 2 | Motility and viability of retrieved sperm

Sperm source	Motility (%)		Vital stain (% viable)	
	Fresh	Thawed	Fresh	Thawed
Testis	5±3.6	0.2±1	86±5	46±12
Epididymis	22±18	7±0.7	57±20	24±13
Vas deferens	71±16	38±13	91±6	51±11

Values are mean±SD. Bachtell *et al.*[11] The relative viability of human spermatozoa from the vas deferens, epididymis, and testis before and after cryopreservation. *Hum. Reprod.* (1999) **14**, 3048–3051, by permission of the European Society of Human Reproduction and Embryology.

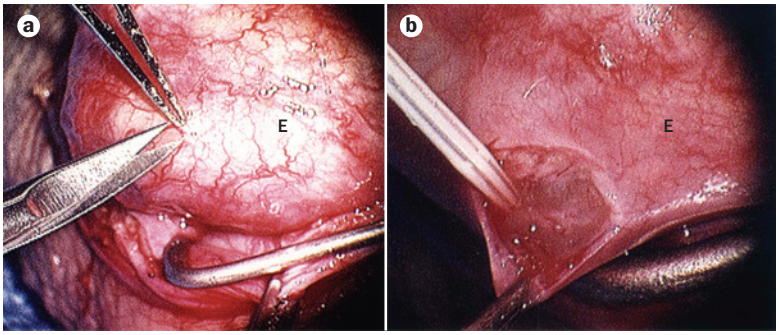

Figure 3 | Microscopic epididymal sperm aspiration (MESA) technique. **a** | The epididymis (E) is exposed through a 1 cm incision. The epididymal tunic is incised overlying a dilated epididymal tubule. **b** | After opening an isolated epididymal tubule under magnification, epididymal fluid is aspirated. With kind permission from Springer Science+Business Media B. V. © Turek, P. J. in *Reproductive Endocrinology and Infertility: Integrating Modern Clinical and Laboratory Practice* (eds Carrell, D. T. & Peterson, C. M.) 453–465 (Springer, 2010).

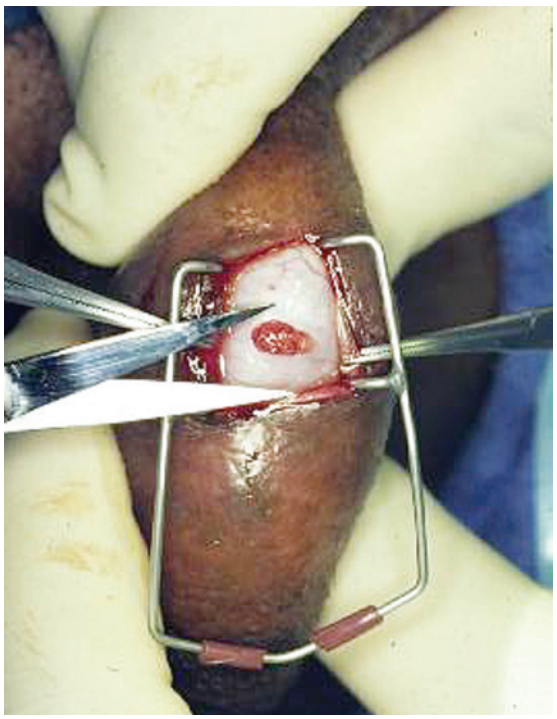

Figure 4 | 'Window' technique for open testicular sperm extraction (TESE). Following a scrotal incision, the tunica vaginalis space is entered. A self-retaining eyelid retractor creates a 'window' into the tunical space. The testis is incised and tissue transected. Multiple different incisions are possible, but the blood supply to the testis can be compromised as the number of incisions increases. With kind permission from Springer Science+Business Media B. V. © Turek, P. J. in *Reproductive Endocrinology and Infertility: Integrating Modern Clinical and Laboratory Practice* (eds Carrell, D. T. & Peterson, C. M.) 453–465 (Springer, 2010).

or opposite testicle as needed.[18] Simple pressure applied to the aspiration site is sufficient for haemostasis. The recovery period is 24 h, with complications that include bleeding (1%) and infection (1%).

As with TESA, TESE can be performed coincident with oocyte harvest or in advance with sperm cryopreservation. The percutaneous biopsy technique involves stabilization of the testicle as described with TESA. However, instead of a using a butterfly needle, a large gauge (16–18 gauge) needle or Biopty® gun (Bard Urological, USA) loaded with a 14 gauge needle is used to obtain core biopsies of the testicle. Aided by a scrotal skin puncture by scalpel, the Biopty® needle is advanced through the skin and into the testicular tunica albuginea either at the lower or upper pole of the testicle and is directed parallel to the long axis of the testicle. After firing the Biopty® gun, the tissue core is placed in culture medium for processing. The main risk with testis tissue retrieval by Biopty® gun is haematoma, which occurs in 1–5% of cases when assessed by ultrasonography.[19]

Open surgical testicular biopsy is another technique for retrieving sperm in patients with obstructive azoospermia (Figure 4). This procedure is generally performed through a 'window' technique in which the testicle and scrotum are stabilized and the scrotal skin incised with a 1 cm incision.[20] The tunica vaginalis is opened and the tunica albuginea is incised parallel to the visible surface vessels. Testicular tissue is excised and further processed for ICSI. The tunica albuginea and scrotum are closed with absorbable sutures. The recovery period is 24–48 h and procedural risks are low but include infection (<1%), bruising, and bleeding (<5%).

In studies that have examined intratesticular bleeding after testicular sperm retrieval, the risk seems to be higher with open compared with percutaneous biopsy techniques (29% versus 7%).[21] However, a more worrisome adverse effect of open biopsy techniques is reduction in testosterone hormone production after large or repeated procedures. This reduction is probably caused by the removal of larger amounts of testis tissue containing both sperm-producing and hormone-producing cells with open surgical techniques (TESE) compared with percutaneous techniques (TESA).

Nonobstructive azoospermia

Nonobstructive azoospermia underlies most cases of azoospermia. Physical examination typically reveals testicular atrophy and hormonal analysis demonstrates elevated FSH levels. Unlike the findings from obstructive azoospermia, only half of patients with nonobstructive azoospermia will have usable testicular sperm for ICSI.[22,23] Furthermore, clinical parameters such as testicle size, serum hormone levels, and testis biopsy histology do not accurately predict whether a testicular procedure will yield sperm.[22–26]

In addition to the overall decreased yield of sperm in nonobstructive azoospermia compared with obstructive azoospermia, it is now clear that sperm production in nonobstructive azoospermia testes can be focal in nature.[27] This fact makes TESA less effective than TESE procedures for successful sperm retrieval. However, even the more invasive TESE procedures can fail to find sperm if performed in a blind or random fashion. To address the problem of focal sperm production, several strategies have been developed to localize sperm in the testes of men with nonobstructive azoospermia while minimizing testicular damage.

Multibiopsy TESE

TESE is the most frequently used approach in cases of nonobstructive azoospermia. The multibiopsy TESE technique was developed in 1997 to maximize successful sperm retrieval by increasing sample numbers. Compared with the single tunical incision used in obstructive azoospermia, multibiopsy TESE involves several tunical incisions with tissue extraction (up to 15 times) until sufficient sperm is obtained.[22] This procedure is often performed in advance of ICSI, and sperm is frozen and later thawed for use.[25] This approach successfully retrieves sperm in ~50% of nonobstructive azoospermia cases, but is considered to be the most invasive option, with the highest risk of damage to the testicle.

Microdissection TESE

First described in 1998, microdissection TESE was developed from the observation that seminiferous tubules with active spermatogenesis appear larger and more opaque under optical magnification than those without active production.[28] Procedurally, the testicle is exposed as in a standard TESE. However, only a single, large, equatorial, or longitudinal incision is made through the tunica albuginea over the full width or length of the testicle, through which the testicular parenchyma is entirely extruded (Figure 5). Exposed seminiferous tubules are examined with microscopy and larger tubules are selectively biopsied until sufficient sperm is collected. Prominent vasculature can be identified and avoided with magnification.

The largest prospective studies comparing microdissection TESE with traditional or multibiopsy TESE demonstrated an absolute sperm retrieval rate advantage of about 15–20% with microdissection.[26,29–31] A subgroup analysis suggested that microdissection TESE lacks superiority to conventional TESE in cases of maturation arrest as seminiferous tubules tend to be of uniform size, reducing the ability to optically discriminate active tubules.[30,32] A systematic review of seven published microdissection studies confirmed that microdissection TESE performs better than conventional TESE only in azoospermia cases of Sertoli-cell-only histology, in which tubules containing active spermatogenesis are more easily differentiated from surrounding germ-cell-free tubules.[18]

An important safety issue in men with nonobstructive azoospermia is hypogonadism after testicular procedures. This complication might necessitate permanent testosterone replacement, and can be a substantial life-long medical burden for men of reproductive age. It has been reported that if fewer TESE biopsy samples are taken, there is a less-pronounced postoperative testosterone decrease.[32] Even with the precise tissue dissection that it offers, microdissection TESE is also associated with measurable effects on androgen balance in many cases.[33–35] Among studies in which patients are followed after microdissection TESE in experienced hands, serum testosterone levels recover to baseline in only 50–85% of patients after 1 year.[33–36] Thus, the magnitude of the effect of open testicular biopsies on testosterone production and testicular atrophy is well-defined and forms the basis for recommending that testicular sperm retrieval procedures be performed no sooner than 6 months apart, as structural changes tend to resolve over this time.[35] These findings have also stimulated the development of further testis-sparing strategies for sperm retrieval.

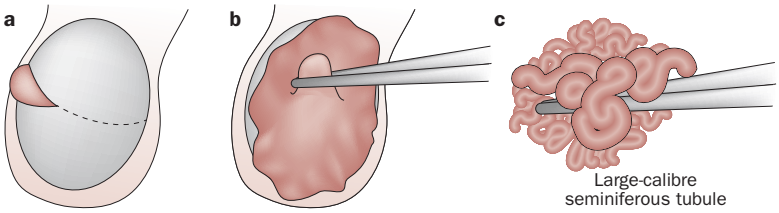

Figure 5 | Microdissection testicular sperm extraction (TESE). **a** | After fully exposing the testicle, a large equatorial or longitudinal incision is made in the tunica albuginea. **b** | The testis parenchyma is fully extruded and **c** | is inspected for large, opaque seminiferous tubules, which are preferentially biopsied as they are more likely to contain sperm.

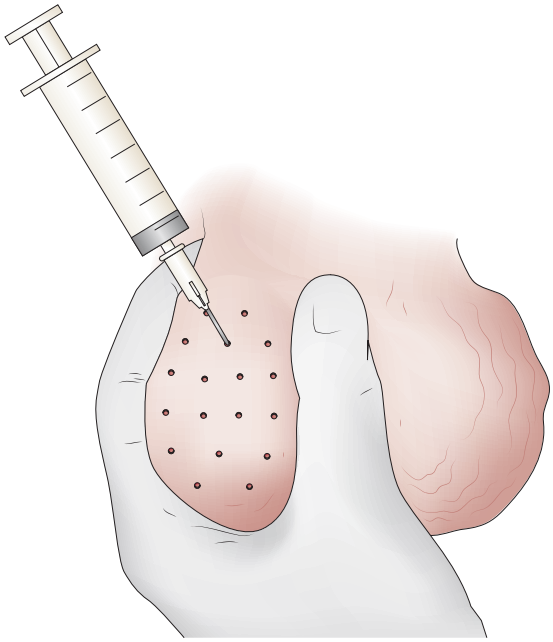

Figure 6 | Fine needle aspiration (FNA) mapping technique. With the scrotal skin stretched taut over the testis, FNA of testis tissue is performed in a systematic, templated way. Aspirated samples are smeared onto slides, fixed, stained, and inspected cytologically for germ cells and sperm.

Fine-needle aspiration map-guided TESE

First reported in 1997, fine-needle aspiration (FNA) map-guided TESE is a two-step procedure that localizes and retrieves foci of sperm in the testes.[37] After sperm is first identified through nonsurgical FNA testicular mapping, sperm retrieval follows and is guided by the map findings (Figure 6). This procedure results in a very high chance of successful sperm retrieval and minimizes invasiveness.[38]

Performed under local anaesthesia, FNA mapping involves taking a template-guided set of aspirated testis tissue samples, ranging in number from four per testicle in a simple map to 18 per testicle in a compound map. Aspirated seminiferous tubules undergo routine Papanicolaou staining and are examined by a

Box 1 | Laboratory effort required for sperm retrieval[53]

- TESA requires 1–2 sperm search man-hours
- Simple TESE requires 2–4 sperm search man-hours
- Microdissection TESE requires 4–6+ sperm search man-hours

Abbreviations: TESA, testicular sperm aspiration; TESE, testicular sperm extraction.

cytopathologist for the presence of sperm.[39] The likelihood of finding testicular sperm increases with sample number, but appears to reach a plateau at approximately 60% detection rate with ≥15 samples.[37] Patient recovery is less than 24 h and an average of two pain pills are taken postprocedure.[27]

If sperm are found on FNA mapping, the couple is counselled to proceed with sperm retrieval and ICSI. The method of testis sperm extraction is then governed by the findings from the mapping procedure. In a series of 159 mapped nonobstructive azoospermia cases, 44% of patients needed TESA, 33% required TESE, and 23% underwent microdissection TESE to retrieve sufficient sperm for all eggs at ICSI.[40] In addition, all microdissection cases in this series were unilateral and involved sperm retrieval from only one testicle. Overall, sufficient sperm was obtained in 95% of cases (100% of TESA and TESE cases and 80% of microdissection TESE cases).[40] In addition, by curtailing the use of microdissection in nonobstructive azoospermia cases, this strategy could theoretically limit postsurgical hypogonadism in this at-risk population. Indeed, the safety profile of the FNA approach has been confirmed in both human and animal studies.[27,41,42]

Comparative outcomes and expectations

A Cochrane review of the published literature on techniques of sperm retrieval found a lack of randomized controlled trials from which to make recommendations for one technique over another. The only recommendation made was to select the least invasive and simplest technique for sperm retrieval whenever possible.[4] A meta-analysis of available nonrandomized data comparing ICSI results ($n = 1{,}103$ cycles) from men with obstructive versus nonobstructive azoospermia found significantly worse outcomes in cases of nonobstructive azoospermia:[43] in a fixed effects model analysis, obstructive azoospermia cases were associated with significantly higher normal fertilization rates (RR 1.18; 95% CI 1.13–1.23) and clinical pregnancy rates (RR 1.36; 95% CI 1.10–1.69) than nonobstructive azoospermia cases. A nonsignificant increase in ongoing pregnancy rates was also detected between these groups (RR 1.19; 95% CI 0.87–1.61). No differences in implantation rates (RR 1.01; 95% CI 0.87–1.61) or miscarriage rates (RR 0.84; 95% CI 0.48–1.48) were observed between these two male factor groups. The findings from this meta-analysis seem to be consistent with the Cochrane recommendations.

Laboratory effort and expectations surrounding surgical sperm retrieval procedures also deserve a mention here. To achieve excellent outcomes, clinical care is carefully timed and orchestrated between procedures on both partners. In nonobstructive azoospermia, andrology laboratory effort is especially important to procedural success. Regarding the technical effort needed to find surgically retrieved sperm, the use of a sliding scale for 'minimal recommended search time' in the laboratory is recommended to ensure adequate outcomes (Box 1). By aligning effort with procedural complexity, laboratory staff are better-prepared for the task at hand. In addition, given that testicular sperm motility generally remains stable with incubation *in vitro* for at least 24 h after retrieval, the delayed use of testicular sperm retrieved in advance of ICSI is now commonly undertaken to simplify the procedural timing for both partners.[44]

Retrieved sperm DNA damage

With the advent of ICSI, the clinical acumen of embryologists has effectively overcome evolutionarily proven physiological selection barriers to fertilization. For this reason, birth, neonatal, and longitudinal developmental outcomes of ICSI offspring have been tracked extensively over the past 20 years. In the past decade, it has become clear that the integrity of sperm DNA and chromatin structure correlates to successful pregnancies in both natural and assisted reproductive settings.[45–47] In addition, sperm DNA integrity is not predictable from descriptive semen parameters, as normal sperm motility and morphology are not guarantees of normal sperm DNA structure.[47,48] Currently, the spotlight that has been focused on the DNA integrity of ejaculated sperm is casting new light on that of surgically retrieved sperm.

Given our current understanding of the pathology of sperm DNA fragmentation, the anatomical location of harvested sperm (testicle, epididymis, or ejaculate) might influence sperm DNA integrity and reproductive outcomes. Sperm DNA damage is thought to occur both during and after spermatogenesis. One source of DNA fragmentation is programmed apoptosis during the late stages of sperm production in association with Sertoli cells.[47] Sperm DNA damage might also be incurred during post-testicular transport and storage within the epididymis and beyond, mainly due to free radical oxidative damage.[47–49] On the basis of these mechanisms, it has been theorized that epididymal or ejaculated sperm could have increased DNA damage compared with testicular sperm.

A small but important clinical study by Greco *et al.*[50] first brought this issue to light. In 18 infertile men whose partners had failed to conceive with ICSI using ejaculated sperm (which showed DNA damage in >15% of spermatozoa), this nonrandomized study compared subsequent ICSI outcomes using first ejaculated sperm and then testicular sperm. In general, testicular sperm was observed to have lower rates of DNA damage (<6%) than ejaculated sperm in individual patients, a finding that has since been confirmed by other studies.[51] In addition, although there were no differences in normal fertilization and cleavage rates at ICSI using either sperm source, pregnancy rates were higher in the TESE–ICSI group than the ejaculated sperm group. Notably, the

study was underpowered to determine the significance of this outcome. Since then, the use of fresh TESE sperm in men with high rates of DNA fragmentation in ejaculated sperm has become more popular, despite the absence of randomized controlled data to suggest well-defined benefits in either ongoing pregnancy or live birth rates.

Three important caveats should be considered when recommending TESE sperm in the setting of DNA-damaged ejaculated sperm. The first is that there is no indication to use TESE sperm in cases of failed IVF or ICSI with ejaculated sperm with normal DNA fragmentation rates or with sperm with normal semen parameters but unexamined for DNA fragmentation. Second, no evidence suggests that TESE sperm should be used instead of ejaculated sperm in cases of unexamined, severely oligospermic semen samples. Third, a genetic trade-off exists in using TESE sperm instead of ejaculated sperm in that testicular sperm has chromosomal aneuploidy rates that are significantly higher (12.4% versus 5.7%) than ejaculated sperm from the same individuals.[51] Whether or not new sperm selection methods, such as evaluation of organellar morphology or hyaluronic acid binding, will improve sperm selection in such cases is yet to be determined.[45]

Sperm cryopreservation

The first pregnancy achieved using cryopreserved sperm was reported in 1953.[52] Since then, cryopreservation of ejaculated and even surgically retrieved sperm has become routine practice in fertility centres throughout the world. Banking surgically retrieved sperm adds convenience to the timing of costly and effort-intensive ICSI procedures for both partners. It also allows for multiple rounds of ICSI after a single sperm retrieval procedure.[12]

The cryobiological behaviour of sperm from various anatomical sites within the reproductive tract is well defined.[11] In a study that reported the effects of cryopreservation on the relative viability of sperm harvested from the vas deferens, epididymis, and testis, several fundamental observations were made. First, regardless of anatomical source, all fully developed sperm tolerate the freeze–thaw process similarly, in that about half of the initial population of viable sperm survives after thaw. Second, the recovery of sperm motility after thaw depends largely on the anatomical source of the sperm (Table 2). In general, vasal sperm has better recovery of motility than epididymal sperm, which in turn has better recovery than testicular sperm. Finally, although both fresh and thawed testicular sperm have very low motility compared with their vasal or epididymal counterparts, they have comparable levels of viability, making testicular sperm a reasonable choice for ICSI.

Epididymal sperm

The question of whether there is a difference in pregnancy outcomes with fresh compared with frozen–thawed surgically retrieved epididymal sperm is a difficult one to answer, again mainly due to the lack of robust randomized controlled trials addressing this topic. In an elegant study of repeated measures design, the same couples underwent sequential ICSI cycles using fresh epididymal and then frozen–thawed (motile) epididymal sperm; no differences in oocyte fertilization, embryo quality, or pregnancy rates were noted.[16] This conclusion has been supported by a meta-analysis.[43]

Testicular sperm

The clinical performance of fresh and frozen–thawed testicular sperm has also been examined. A meta-analysis examining the ICSI outcomes of fresh and frozen–thawed testis sperm has suggested that fertilization, clinical pregnancy, and ongoing clinical pregnancy rates do not differ between these two types of sperm.[43] However, a significant decrease in implantation rates (RR 1.75; 95% CI 1.10–2.80) was observed with frozen–thawed compared with fresh testis sperm, possibly reflecting differences in the ability to select viable sperm between these groups. In a study of DNA damage occurring with cryopreservation of testicular sperm, DNA fragmentation rates before and after cryopreservation did not differ in either fertile men or in those with obstructive azoospermia.[49] In men with nonobstructive azoospermia, no significant difference in pregnancy outcome was found with ICSI using fresh compared with frozen–thawed sperm.[43] Guided by this data, each centre should develop its own algorithm regarding the timing of sperm retrieval and the type of sperm that best suits its capability, needs, and goals.

Conclusions

From an evidence basis, many different surgical sperm retrieval techniques exist and no single procedure is optimal for either obstructive or nonobstructive azoospermia. Although it is not difficult to retrieve sperm when obstruction is present, it can be very difficult to find sperm in nonobstructive azoospermia, as sperm production can be patchy or focal. Unfortunately, no randomized controlled trials have compared the efficacy of current sperm retrieval strategies for nonobstructive azoospermia; each technique has its own strengths and limitations. However, given that ICSI is not always successful, it behoves reproductive urologists to develop and apply retrieval techniques that are reliable, of low morbidity, and that harvest sufficient sperm to enable multiple ICSI attempts without repetition. Through improved understanding of sperm cryobiological behaviour and the relative reproductive competence of sperm from different anatomical sources, retrieval procedures can be individualized and optimized to achieve these goals.

Review criteria

We searched for original articles focusing on the treatment of obstructive and nonobstructive azoospermia in Medline and PubMed published between 1990 and 2013. The search terms we used were "sperm retrieval", "testicular sperm", "epididymal sperm", "vasal sperm", "nonobstructive azoospermia", and "obstructive azoospermia". All papers identified were English-language full-text papers. We also searched the reference lists of identified articles for further papers.

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Author contributions
Both authors researched the data for the article, provided a substantial contribution to discussions of the content and contributed to writing the article and to review and/or editing of the manuscript before submission.