

Which is the best sperm retrieval technique for non-obstructive azoospermia? A systematic review

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Our objective was to establish which is the best sperm retrieval technique in non-obstructive azoospermia based on the available evidence. 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. Three outcome measures were assessed for the sperm retrieval techniques: sperm retrieval rate (SRR), complications and live birth rate. Twenty-four descriptive studies reporting on the results of testicular sperm extraction (TESE) were encountered. Seven controlled studies that compared microdissection (MD) TESE with conventional TESE and seven controlled studies comparing fine needle testicular aspiration (FNA) with TESE were identified. The mean SRR for TESE was 49.5% (95% CI 49.0–49.9). TESE with multiple biopsies results in a higher SRR than FNA especially in cases of Sertoli-cell-only (SCO) syndrome and maturation arrest. Current evidence suggests that MD performs better than conventional TESE only in cases of SCO where tubules containing active focus of spermatogenesis can be identified. MD appears to be the safest technique regarding post-operative complications followed by FNA. Only three studies could be identified concerning the influence of the sperm retrieval technique on clinical pregnancy and live birth rate, hence no definitive conclusions can be made. However, so far there appears to be no impact of the technique itself on success rates.

Keywords: non obstructive azoospermia; sperm retrieval; testicular sperm extraction

Introduction

Azoospermia defined as the absence of spermatozoa in the ejaculate after assessment of centrifuged semen on at least two occasions, is observed in 1% of the general population and in 10–15% of infertile men (Willott, 1982; Jarow *et al.*, 1989). The introduction of intracytoplasmic sperm injection (ICSI) in 1992 (Palermo *et al.*, 1992; Van Steirteghem *et al.*, 1993) offered a novel opportunity for parenthood to these couples. The first pregnancies reported after fertilization by ICSI with testicular sperm in men with obstructive azoospermia (OA) were published in 1993 (Craft *et al.*, 1993; Schoysman *et al.*, 1993), although the first pregnancy using epididymal sperm had been reported a decade earlier (Temple-Smith *et al.*, 1985). Testicular sperm extraction (TESE) has been described for the first time in 1994 (Devroey *et al.*, 1994). The use of TESE in non-obstructive azoospermia (NOA) has been reported subsequently (Devroey *et al.*, 1995; Tournaye *et al.*, 1995).

NOA results from a testicular failure. This problem affects 10% of infertile men and is diagnosed in 60% of azoospermic men (Jarow *et al.*, 1989; Matsumiya *et al.*, 1994). Aetiologies for testicular failure include genetic disorders such as sexual

chromosomal abnormalities, translocations and microdeletions of the Y chromosome, cryptorchidism, testicular torsion, radiation and toxins (Jarow *et al.*, 1989; Palermo *et al.*, 1999; Ezech, 2000; Raman and Schlegel, 2003).

Testicular spermatozoa can be retrieved in some NOA men despite the absence of ejaculated spermatozoa in their semen, because of the existence of isolated foci of active spermatogenesis. Early reports on TESE including a limited number of patients raised optimism because of a high sperm retrieval rate (SRR) (80%) (Devroey *et al.*, 1995; Silber, 1995; Tournaye *et al.*, 1996), however, lower recovery rates (around 50%) were observed in subsequent larger series (Tournaye *et al.*, 1997a; Gil-Salmon *et al.*, 1998).

Current recommendations on the diagnosis of NOA dictate that it should only be based on histopathological findings since clinical and endocrine parameters cannot accurately distinguish between OA and NOA (Tournaye *et al.*, 1995; Devroey, 1998; Schoor *et al.*, 2002; Tournaye, 2002; McLachlan *et al.*, 2007). The former represents an important issue since sperm can be retrieved in almost all cases of OA, but only in 50% of NOA when no preliminary selection of patients on the basis of histopathology has been performed (Tournaye *et al.*, 1997a). Hence,

only patients with histological confirmation of defective spermatogenesis should be included in clinical trials aiming at assessing the efficiency of surgical techniques to retrieve testicular sperm in NOA.

An ideal surgical technique would enable, with a minimal trauma to the testis, the retrieval of a sufficient amount of motile spermatozoa to inject all available oocytes and to cryopreserve the remainder in case if a further attempt is needed. Nevertheless, none of the currently available techniques fulfils these criteria.

TESE with multiple biopsies was proposed as a way to enhance SRR of a single biopsy given that in many cases of NOA only isolated regions of spermatogenic tissue are present (Tournaye *et al.*, 1995, 1996; Hauser *et al.*, 1998). Nonetheless, the removal of large samples of testicular tissue can lead in some cases to testicular atrophy (Schlegel *et al.*, 1997; Tash and Schlegel, 2001). On the other hand, fine needle aspiration (FNA) offers a less invasive alternative, however, its effectiveness in NOA has been questioned (Friedler *et al.*, 1997; Ezech *et al.*, 1998; Rosenlund *et al.*, 1998; Tournaye, 1999). According to three prospective controlled studies, TESE is more effective than FNA (Friedler *et al.*, 1997; Tournaye *et al.*, 1997a; Ezech *et al.*, 1998), yet a recently updated Cochrane database review concluded that there is insufficient evidence to recommend any particular surgical technique (Van Peperstraten *et al.*, 2006).

Microdissection (MD) TESE was developed to combine the advantages of a less invasive approach with an open excisional biopsy, hence minimizing testicular trauma by identifying the zones of active spermatogenesis through optical magnification (Schlegel, 1999). Although most of the reports reveal promising results (Schlegel, 1999; Amer *et al.*, 2000; Tsujimura *et al.*, 2004; Ramasamy *et al.*, 2005), to date no randomized trial has compared the effectiveness and safety of MD with conventional TESE.

In recent years, the addition of non-invasive exams such as colour Doppler ultrasound to guide FNA or TESE through the identification of regions with higher vascularization showing active spermatogenesis has been assessed (Foresta *et al.*, 1998; Har-Toov *et al.*, 2004; Tunc *et al.*, 2005; Herwig *et al.*, 2007).

The aim of this review is to establish through a systematic review of the literature, which is the best surgical technique for sperm retrieval in NOA men.

Search strategy

A computer-based search on the databases of Medline, Embase and Cochrane Menstrual Disorders and Subfertility group using the following search terms was conducted: non-obstructive azoospermia, sperm retrieval, testicular sperm extraction, fine needle aspiration, testicular sperm aspiration, microdissection TESE. The search aimed to identify randomized, observational and descriptive studies evaluating techniques for sperm retrieval in NOA men. Since no randomized trials were found, only controlled, observational and descriptive studies are discussed in this review.

Outcome measures

The primary outcome measure of this systematic review was the SRR since in NOA the finding of spermatozoa represents the

most relevant step before treatment. The secondary outcome measures were complications and live birth rate of the different techniques.

Sperm retrieval rate

In NOA, the finding of testicular spermatozoa constitutes a key element for successful treatment as only in half of these patients sperm can be found (Gil-Salom *et al.*, 1998). Most of the studies define success as the recovery of at least one spermatozoon; however, others also consider morphology and motility of the retrieved sperm as relevant parameters (Gil-Salom *et al.*, 1998).

The negative psychological impact of a failed procedure to recover testicular sperm has led to an important effort on the evaluation of prognostic factors (Tournaye *et al.*, 1996; Ezech *et al.*, 1999; Tunc *et al.*, 2006). Nevertheless, to date, only histopathology using the classification outlined by Levin (1979) has been shown to predict the probability of finding sperm (sensitivity of 86% and specificity of 93%), especially in Sertoli-cell-only (SCO) patients (accuracy 0.83), but not in the cases showing maturation arrest (MA) (accuracy 0.55) (Tournaye *et al.*, 1997b). Other factors that have shown an association with the probability of recovering sperm include history of orchitis, testicular volume, age at orchiopexy for cryptorchidism, Klinefelter's syndrome, the absence of spermatozoa in diagnostic testicular biopsy and AZFb microdeletions (Silber *et al.*, 1997; Brandell *et al.*, 1998; Gil-Salom *et al.*, 1998; Krausz *et al.*, 2000).

The importance of establishing a uniform definition of the pathological status of testicular biopsies has been recently highlighted and a new classification proposed: (i) Normal testicular biopsy (ii) Hypospermatogenesis (iii) Germ cell arrest (iv) SCO appearance (v) Seminiferous tubule hyalinization (vi) Carcinoma *in situ* and (vii) Immature testis (McLachlan *et al.*, 2007). This classification will enable an accurate comparison of the results from differing studies, which represents the major limitation of the available evidence.

Three techniques are currently available for testicular sperm retrieval, which are independently analysed including studies reporting on SRRs. The most important limitations of the available evidence are the inclusion of patients without histological confirmation of testicular failure and the lack of adequate control groups. In addition, the design of controlled studies in which two techniques are performed on the same patient is limited since biopsies may randomly sample different regions of the testis with different patterns, hence, the same patient could be characterized differently on different biopsies.

Testicular sperm extraction

The use of testicular sperm was initially introduced as an alternative to epididymal sperm for cases of OA without the possibility of surgical repair (Schoysman *et al.*, 1993) and later implemented in NOA as ICSI enabled the achievement of pregnancies in these couples as well (Devroey *et al.*, 1995; Tournaye *et al.*, 1995).

TESE is currently the most frequently used technique in NOA men with a mean SRR weighed by sample size of 49.5% (95% CI 49.0–49.9) (Table 1). A recent review on TESE including

Table 1: Descriptive studies evaluating TESE results in NOA

| Study | Unit of study (n) | Total no. of biopsies/testes | SRR (%) | SRR (%) according to histopathology | Fertilization rate (%) | Pregnancy rate cycle/ET (%) |
|---------------------------------|---|------------------------------|---------|---|------------------------|-----------------------------|
| Devroey <i>et al.</i> (1995) | 15 patients | – | 86.6 | – | 47.8 | –/25 |
| Kahraman <i>et al.</i> (1996) | 29 patients | – | 48.2 | – | 38.6 | 20.7/54.5 |
| Mulhall <i>et al.</i> (1997) | 30 patients | – | 70 | 50 SCO 75 MA | – | – |
| Schlegel <i>et al.</i> (1997) | 16 patients | – | 62 | – | 52 | 31/– |
| Silber <i>et al.</i> (1997) | 45 patients | 4–8 | 51.1 | – | – | –/55 |
| Witt <i>et al.</i> (1997) | 20 testicles | 4 | 85 | – | – | – |
| Ostad <i>et al.</i> (1998) | 81 patients | 3–4 | 58 | – | – | – |
| Gil-Salmon <i>et al.</i> (1998) | 154 patients | 4 | 41 | 33 SCO 54 MA 10 TE | 55 | 28/31 |
| Hauser <i>et al.</i> (1998) | 29 patients | 3 | 62.1 | – | 54.5 | –/33.3 |
| Koci <i>et al.</i> (1998) | 25 patients | – | 48 | – | – | – |
| Sobek <i>et al.</i> (1998) | 27 patients | – | 56.6 | – | 30 | –/27 |
| Ben-Yosef <i>et al.</i> (1999) | 55 patients | 3 | 60 | – | 59 | –/25 |
| Su <i>et al.</i> (1999) | 75 patients | – | 58 | 79 Hypospermatogenesis 47 MA 24 SCO | 61 | –/55 |
| Kitamura <i>et al.</i> (2000) | 44 patients | – | 72.7 | 30 SCO | – | –/46.9 |
| Ng <i>et al.</i> (2000) | 26 patients | 1 | 46.2 | – | 63.3 | 14.3/– |
| Seo and Ko (2001) | 178 patients | 3–4 | 52.8 | 16.3 SCO 62.5 MA 89.2 Hypospermatogenesis | – | – |
| Damani <i>et al.</i> (2002) | 23 patients | 3–4 | 65.2 | – | 65.2 | 30.8/– |
| Friedler <i>et al.</i> (2002b) | 123 patients | – | 41 | 70.8 Hypospermatogenesis 25.9 SCO 43.7 MA 41.7 TE | – | –/32.4 |
| Meseguer <i>et al.</i> , 2003 | 12 patients | 3 | 41.6 | 18 SCO 100 Hypospermatogenesis 100 MA (1/1) | 68 | –/14.3 |
| Wood <i>et al.</i> (2003) | 21 patients | 1 | 61 | – | – | – |
| Vernaev <i>et al.</i> (2004) | 79 patients History of orchidopexy | 1–4 | 51.9 | 48 SCO 70 MA 20 Sclerosis and atrophy | 50.5 | –/34 |
| Okada <i>et al.</i> (2005) | 51 patients non-mosaic Klinefelter syndrome | – | 50.9 | – | – | –/46.1 |
| Tunc <i>et al.</i> (2006) | 52 patients | – | 59.6 | – | – | – |
| Vernaev <i>et al.</i> (2006) | 628 patients | 1–4 | 41.6 | 38.7 SCO 49.7 MA 38.8 Sclerosis and atrophy | – | – |

SRR, sperm retrieval rate; SCO, Sertoli cell only; MA, maturation arrest.

also observational studies described a mean successful rate of 52% (Colpi *et al.*, 2005).

In cases associated with cryptorchidism, a significantly higher success rate than unexplained NOA has been reported (74 versus 58%) (Raman and Schlegel, 2003) (51.9%; 95% CI 40.9–62.9 versus 33.3%; 95% CI 27.0–39.7) (Vernaev *et al.*, 2004), which could, however, be a consequence of the inclusion of patients with retractile testis rather than cryptorchidism.

The most appropriate number of biopsies to be taken remains controversial. Single testicular biopsy has been advocated as the best technique based on the finding that spermatogenesis in NOA is multifocal (Silber *et al.*, 1997); hence a single large sample would be representative of the whole testis (Roosen-Runge, 1956; Steinberg and Tjioe, 1968). Nevertheless, this approach was refuted by other studies that found a patchy distribution of regions with minimal spermatogenesis throughout

the testis (Devroey *et al.*, 1995; Gil Salom *et al.*, 1995; Tournaye *et al.*, 1996; Hauser *et al.*, 1998). Therefore, taking multiple samples from different sites of the testis could increase the chance of finding a focus of active sperm production. In addition, it has been shown that the number of biopsies required to retrieve motile sperm is significantly higher in cases of MA and SCO compared with patients with normal spermatogenesis (4.2 ± 4.5 , 2.8 ± 2.5 , 1.2 ± 0.5 , respectively) (Tournaye *et al.*, 1996).

Only one randomized study has so far compared TESE using a single extended incision with multiple incisions in a group of 89 men in which the first incision failed to find spermatozoa (Fahmy *et al.*, 2000). Both groups had similar mean weights of testicular tissue removed. SRRs were comparable between both techniques (29.5% for single biopsy versus 26.7% for multiple biopsies). Nonetheless, no details were given on the methodology used for allocation.

On the other hand, the evidence provided by observational studies favours multiple biopsies. In a study where three testicular biopsies were taken from identical locations in 29 men spermatozoa were found in 28 testes, in one location in 8 testes (28.6%), in two locations in 5 testes (17.9%) and in three locations in 15 cases (53.6%) (Hauser *et al.*, 1998). Further analysis of these data revealed that if only one biopsy had been performed instead of three between 18 and 32% of the sperm found in different locations of the testis would have been missed (Hauser *et al.*, 1998). Nevertheless, these figures derive from a rather theoretical estimation rather than a sound difference between both approaches. A larger observational study including 316 NOA men compared single bilateral testicular biopsy ($n = 216$) with multiple biopsies ($n = 100$) (Amer *et al.*, 1999). The distribution according to histopathological findings was similar in both groups. The multiple biopsy approach enabled a significantly higher SRR compared with single biopsy (49 versus 37.5%) (Amer *et al.*, 1999), however, this difference was only observed in cases of MA (85 versus 58.7%), and mixed pathology (65.4 versus 56.6%), but not in SCO (24.2 versus 23.2%) and tubular sclerosis (25 versus 22.2%) (Amer *et al.*, 1999). Moreover, the percentile incidence of positive TESE raised from 60 to 100% when the number of biopsies increased from two to four.

Taking multiple biopsies could also add a higher chance of finding motile sperm as it was shown that in 35% of the cases only non-motile spermatozoa were found in one location and only motile in others (Hauser *et al.*, 1998).

Two descriptive studies compared sperm recovery rates according to the location of the biopsy reporting contradictory results (Witt *et al.*, 1997; Hauser *et al.*, 1998). Hauser *et al.* (1998) found no advantage of any particular site of the testicle after performing biopsies on the upper pole, midline and proximal pole in 29 men (55 testicles), whereas Witt *et al.* (1997) concluded from a smaller series (20 testicles) that the midline portion of the testis enabled the highest retrieval rate.

Doppler ultrasound has been applied prior to TESE to trace regions with better vascularization (Herwig *et al.*, 2004) finding a higher SRR in areas with good compared with poor vascularity (38 versus 14%) (Tunc *et al.*, 2005). In addition, power Doppler ultrasound was employed to calculate a testicular vascular index, which showed a sensitivity of 47.3% and specificity of 89.8% for the finding of sperm (Har-Toov *et al.*, 2004). High-resolution

colour Doppler ultrasound performed prior to TESE focused on the evaluation of tissue perfusion has recently revealed a positive correlation with the quantity and quality of the isolated sperm (Herwig *et al.*, 2007). Hence, these techniques could reduce the number of biopsies required to retrieve sperm, thus minimizing testicular damage. Nevertheless, more studies are required to establish the added value of these non-invasive tests.

The main drawbacks of TESE are the loss of a significant amount of testicular tissue and a disruption in the blood supply of the tunica albuginea leading to fibrosis and the possible establishment of an autoimmune response. An impaired testosterone synthesis has also been documented after TESE in a small patient population (Manning *et al.*, 1998).

Despite these limitations, repeated TESE procedures yield high recovery rates (Friedler *et al.*, 2002a; Kamal *et al.*, 2004; Vernaev *et al.*, 2006). A retrospective study including only men in whom sperm had been found on a first TESE reported SRRs of 74.7 and 82.3% after a second and third attempt, respectively (Vernaev *et al.*, 2006). The best moment to perform a second biopsy in patients that require a repeated TESE remains controversial. Amer *et al.* (1999) evaluated 27 patients who underwent a second TESE after 1–24 months finding no significant difference between the groups who had a second biopsy before or after 3 months from the first TESE (75 versus 94.7%). Similar SRRs were also reported on a larger series (156 biopsies) when the second TESE was performed before or after 6 months from the first procedure (82.7 versus 76.5%) (Vernaev *et al.*, 2006). On the contrary, Schlegel and Su (1997) described a higher retrieval rate when the second biopsy was performed after 6 months (80 versus 25%).

Microdissection TESE

The identification of areas in which spermatogenesis still occurs represents the background for the addition of magnification to TESE. Individual seminiferous tubules can be seen under the microscope at 25 \times power allowing the identification of larger, whitish and opaque tubules in which spermatogenesis is active in opposition to tubules where no sperm production occurs (Schlegel, 1999). This strategy could facilitate the removal of smaller amounts of testicular tissue, which becomes crucial in the presence of testicular atrophy. In addition, the identification of avascular regions for the opening of the tunica albuginea could minimize the chances of vascular injury.

The first report on this technique compared 22 patients whom underwent standard multiple biopsy with a group of 27 men undergoing MD (Schlegel, 1999). Although the author describes a significant improvement on the SRR when MD had been performed (63 versus 45%), no data were given regarding the histology found in these two groups. Moreover, no matching was described between these two groups. A smaller amount of tissue was however excised with the MD approach (9.4 versus 720 mg).

To date, the largest series on MD reported the results of 684 procedures in 563 men achieving a SRR of 61% (Schlegel *et al.*, 2006). A comparative study including 116 men found a significantly higher SRR with the addition of optical magnification compared with conventional TESE (47 versus 30%) (Amer *et al.*, 2000). Nonetheless, that higher success rate was by supposition

only observed for hypospermatogenesis of varying degrees. Only patients with bilateral non-identical histopathology were excluded from analysis, but not eight men who had normal histopathology. Another limitation of this study is that since the biopsy procedure was performed on a diagnosis basis, only one sample was taken on the conventional TESE group and two on the microsurgical.

Okada *et al.* (2002) conducted an additional retrospective study that compared conventional TESE with MD analyzing the results of 98 men (24 conventional TESE and 76 MD). No matching between these groups of patients was reported. A significantly higher SRR was described after MD compared with TESE (44.6 versus 16.7%). Furthermore, only MD enabled the retrieval of spermatozoa in Klinefelter's syndrome patients. Subgroup analysis revealed that only SCO had a significantly higher SRR with MD (33.9 versus 6.3%; $P = 0.04$), but not men with MA (75 versus 37.5%; $P = 0.2$). A possible explanation for this difference is that in cases of MA all tubules are uniform despite the presence of active spermatogenesis as opposed to SCO where the difference in tubules enables the identification of sites of spermatogenesis (Silber, 2000). Nonetheless, a larger study including 435 men undergoing 533 procedures (83 conventional TESE and 460 MD) found a significantly higher SRR only in cases of hypospermatogenesis (81 versus 50%), but not in SCO and MA (Ramasamy *et al.*, 2005). The study design, however, does not enable definitive conclusions since patients on the TESE and MD groups were not matched.

In addition to histopathology, testicular volume has also shown a correlation with MD SRR. A retrospective study found that only men with a testicular volume lower than 10 ml had a better recovery rate with MD compared with TESE (42 versus 27%) (Mulhall

et al., 2005). Both groups were comparable regarding age and histopathological distribution. An additional advantage of MD observed in this study was a lesser need for bilateral biopsy (42 versus 82%) as well as a reduced number of tunical incisions (1.4 ± 0.4 versus 3.2 ± 1.2).

In opposition to these results, Tsujimura *et al.* (2002) reported comparable results with conventional and MD techniques (SRR of 35.1 versus 42.9%, respectively). Both groups had similar age, testicular size, endocrine profiles and histological findings. Operative time was significantly higher on the MD group (146.8 ± 52.3 versus 68.2 ± 24.5 min). Spermatozoa were retrieved in all cases that had only homogeneously thick tubules. On the contrary, no spermatozoa could be recovered when only homogeneously thin tubules were observed.

A small series of 13 men reported a SRR of 48% when MD was applied as a salvage technique immediately after a failed TESE (Okubo *et al.*, 2002). In agreement with these results Tsujimura *et al.* (2006) found comparable results in patients undergoing primary MD (45.7 versus 44%) or after a failed conventional TESE. An important limitation of this study is that 30% of the patients had only a unilateral conventional TESE and in only nine of them multiple biopsies were taken. Nevertheless, this technique was successful in three out of nine patients with a previous bilateral and multiple sample failed TESE. Ramasamy and Schlegel (2007) recently reported the lack of threshold for the number of previous negative biopsies before undergoing MD, although the SRR was significantly lower in patients who underwent three or four biopsies compared with one or two biopsies (23 versus 51%).

Table 2 summarizes the controlled studies comparing SRRs with MD and conventional TESE in NOA.

Table 2: Controlled studies comparing MD with conventional TESE in NOA

| Study | Design | Patients (n) | SRR (%) MD/TESE | Pregnancy rate/ET (%) MD/TESE | Complications (%) MD/TESE |
|--------------------------------|---------------|---|--------------------|----------------------------------|--|
| Schlegel <i>et al.</i> (1999) | Prospective | 22 TESE 27 MD | 63/45 | – | None |
| Amer <i>et al.</i> (2000) | Prospective | 100 one testis TESE and one testis MD | 47/30 | – | 6.7/51.7 Haematoma US after 3 months after surgery 3.3/30 Fibrosis US after 6 months |
| Okada <i>et al.</i> (2002) | Retrospective | 24 TESE | 44.6/16.7 | – | 12/51 Haematoma US after 1 month 0/23 Fibrosis US after 6 months |
| Okubo <i>et al.</i> (2002) | Prospective | 74 MD 17 TESE 13 MD in patients with failed TESE | 24/30.7 | – | – |
| Tsujimura <i>et al.</i> (2002) | Retrospective | 37 TESE 56 MD | 42.9/35.1 | – | – |
| Mulhall <i>et al.</i> (2005) | Retrospective | 44 TESE 48 MD | 45/50 | – | – |
| Ramasamy <i>et al.</i> (2005) | Retrospective | 83 TESE 460 MD | 58/32 | – | 44/80 Haematoma US after 3 months |

SRR, sperm retrieval rate; MD, microdissection; TESE, testicular sperm extraction; ET, embryo transfer; US, ultrasound.

Testicular FNA

FNA was initially used for diagnostic purposes (Huhner, 1928) and later on to establish the likelihood of sperm retrieval on the day of oocyte retrieval for ICSI (Turek *et al.*, 1999; Fasouliotis *et al.*, 2002). The first report of a delivery after ICSI with testicular sperm retrieved through FNA in NOA was published in 1996 (Lewin *et al.*, 1996). Different techniques have been described with variations in the needle diameter (18–21 gauge) and the number of testicular punctures (range 1–6).

The main advantages of this technique are simplicity, low cost, being minimally invasive and that it produces less post-operative pain compared with TESE under local anaesthesia (Tournaye, 1999). Moreover, it has been suggested that FNA could increase the chance of finding a site of active spermatogenesis by reaching deeper testicular sites (Lewin *et al.*, 1999). Nevertheless, most of the controlled studies have shown significantly lower SRR compared with TESE (Friedler *et al.*, 1997; Ezeh *et al.*, 1998; Rosenlund *et al.*, 1998; Tournaye, 1999). An additional disadvantage is that frequently there are no supernumerary spermatozoa to cryopreserve because of the limited number retrieved (Friedler *et al.*, 1997). In fact, a recent prospective study reported a significantly lower number of frozen straws per patient compared with TESE (0.6 ± 1.1 versus 4.4 ± 5.4) (Hauser *et al.*, 2006).

No randomized study has so far compared the SRR of FNA with TESE in NOA, thus current recommendations are only supported by controlled observational studies (Table 3). Nonetheless, most of these controlled studies did not include an histological characterization of the patients whom underwent attempted treatment. The largest observational study so far conducted reported on the results of 87 men undergoing multifocal FNA followed by immediate multifocal TESE on the same testes (Hauser *et al.*, 2006). A significantly lower motile SRR was obtained when only FNA had been performed compared with TESE (3.4 versus 31%). Moreover, a significantly higher quantity of sperm cells, number of locations with spermatogenesis per testis, number of locations with motile sperm cell per testis were reported following TESE compared with testicular sperm aspiration (FNA). Previous observational studies also showed similar results. A significantly lower SRR was reported in a series of 37 NOA patients in whom FNA was performed before TESE using

a 21-gauge butterfly needle (11 versus 43%) (Friedler *et al.*, 1997). In addition, Ezeh *et al.* (1998) found that multiple FNA using a 19-gauge butterfly needle enabled the retrieval of spermatozoa in only 5 men (14%) as opposed to single open biopsy that retrieved spermatozoa in 17 out of 22 patients in whom FNA had failed. Furthermore, Tournaye (1999) documented a significantly lower SRR in 14 patients whom underwent FNA followed by TESE (7.4 versus 64.3%).

A few observational studies have, however, shown comparable results for both FNA and TESE. A retrospective cohort study including 60 patients found a similar motile sperm recovery rate when FNA had been performed compared with TESE (24.5 versus 19.2%, respectively) (Nassar *et al.*, 2001). Moreover, TESE was unsuccessful in nine cycles where also FNA had failed. Both groups had comparable histopathological diagnosis distribution. Although the study conducted by Aridogan *et al.* (2003) also reported similar retrieval rates with TESE and FNA (40.8 versus 39.5%, respectively), this series included OA patients as well as men with severe oligozoospermia. Multiple descriptive studies have additionally shown similar SRRs to TESE (~50%). Lewin *et al.* (1999) found a SRR of 58% in a group of 85 men undergoing FNA. Ten or less spermatozoa were recovered in 46.2% of the cycles, tens of spermatozoa in 40% and hundreds to thousands in 13.8%. The highest recovery rate was observed in hypospermatogenesis (95%) followed by non-mosaic Klinefelter's syndrome (66%), SCO (48.3%) and MA (46.4%). A larger series from the same study group (152 patients; 236 FNA procedures) reported a SRR of 53.8% (Lewin *et al.*, 2000). Two other descriptive studies (Tallarini *et al.*, 2002; Levine *et al.*, 2003) also achieved similar results (SRR 61.5 and 47%, respectively). Moreover, Bibancos *et al.* (2004) recovered motile sperm in 63% of the procedures using a 21-gauge needle. An important limitation of this study is that in 31% of patients a histopathological diagnosis is lacking. Several other descriptive studies found poorer results for this technique (25% SRR) (Belker *et al.*, 1998; Westlander *et al.*, 1999; Qublan *et al.*, 2002).

Subgroup analysis on histopathological diagnosis documented good results in cases of hypospermatogenesis (68.9%), yet extremely low in SCO (2.2%) (Khadra *et al.*, 2003). On the contrary, Hauser *et al.* (2006) found no significant association between

Table 3: Controlled studies comparing FNA with TESE in NOA

| Study | Unit of study (ns) | SRR (%) FNA/TESE | Pregnancy rate/ET (%) FNA/TESE | Complications |
|--------------------------------|---|------------------------|--------------------------------|---|
| Friedler <i>et al.</i> (1997) | 37 patients FNA prior to TESE | 11/43 | –/29 | 1 case of bleeding in FNA group 2 cases of haematoma in TESE |
| Ezeh <i>et al.</i> (1998) | 35 patients FNA prior to TESE | 14/77 | – | – |
| Rosenlund <i>et al.</i> (1998) | 22 patients FNA 19 or 21gauge (G) prior to TESE | 21 G 16.7/50 19G 60/70 | – | – |
| Tournaye <i>et al.</i> (1999) | 14 patients FNA 14 patients TESE | 7.1/64.3 | – | – |
| Nassar <i>et al.</i> (2001) | 49 cycles FNA 26 cycles TESE | 24.5/19.2 | 8.2/11.5 | – |
| Khadra <i>et al.</i> (2003) | 84 patients undergoing FNA. TESE was only performed if FNA failed | 53.6/71.8 | 41.8/39.1 | None |
| Hauser <i>et al.</i> (2006) | 87 patients FNA prior to TESE | 24.1/62.1 | – | – |

ET, embryo transfer; FNA, fine needle aspiration; TESE, testicular sperm extraction.

SRR and the histopathological pattern; however, the highest efficiency for FNA was also observed in the presence of hypospermatogenesis (69%).

As regards to the importance of the needle diameter employed for FNA, Rosenlund *et al.* (1998) observed comparable results to TESE when a 19-gauge needle was used (70 versus 60%). A poorer outcome was, however, shown for the 21-gauge needle (16.7% for FNA versus 50% for TESE). Yet, another study showed that TESE yielded a significantly higher SRR compared with FNA, despite the use of an 18-gauge needle (62.1 versus 24.1%) (Hauser *et al.*, 2006).

FNA has also been proposed as a mapping technique by means of performing multiple punctures prior to TESE (Turek *et al.*, 1999; Meng *et al.*, 2000; Damani *et al.*, 2002) resulting in the retrieval of sperm in 95% of the cycles (Turek *et al.*, 1999). Furthermore, in 20% of the cases TESE was not required. However, this study also included patients who previously had documented spermatogenesis, hence, the high SRR reported does not accurately reflect the chance of sperm retrieval in an unselected group of patients with NOA. FNA mapping has shown a high cyto-histological correlation with multiple biopsies (92%) (Hota and Sasturkar, 2006). Another study (Jad and Turek, 2002) used FNA mapping to select, based on the amount of sites with a positive result, between MD and conventional TESE. Difficult cases, defined as patients in whom only one out of 22 mapped sites had a positive result underwent MD achieving a SRR of 86%. On the other hand, conventional TESE was chosen when two or more positive mapped sites were located reporting a SRR of 98%. No information was, however, given regarding the means by which the diagnosis of NOA had been made.

Large needle percutaneous aspiration biopsy showed good results (60% SRR) irrespectively of testicular volume in a descriptive study (Carpi *et al.*, 2006). Furthermore, the mean dimensions of the tissue were similar to those obtained with open surgical biopsy.

Testicular gun needle biopsy constitutes another percutaneous technique for testicular sperm retrieval, however, only limited evidence is available. Tuuri *et al.* (1998) evaluated the effectiveness of gun needle biopsy in 10 NOA men with positive results in six cases. The authors state that when no spermatozoa were retrieved after the first attempt, two or three additional samples could be taken from other directions through the same hole in the tunica albuginea.

Colour Doppler ultrasound has been proposed as a way to improve FNA results through the identification of areas with better vascularization where foci of spermatogenesis could be found. Foresta *et al.* (1998) designed a semi-quantitative score stratifying patients according to the number of intratesticular vessels in three categories: 0 visible vessels, 1–3 vessels and >3 vessels. When FNA was performed in the sites where blood vessels had been identified mature spermatozoa were recovered in 12 out of 16 testis (Foresta *et al.*, 1998). In addition, another study reported a high sperm retrieval (94%) after ultrasound-guided FNA with minor late complications in 13% of the cases (Belenky *et al.*, 2001). Nevertheless, patients were selected solely based on clinical and endocrinological evaluation, hence, possibly also including cases of OA. Thus, further research is needed in this area to determine if it could enhance FNA results in NOA.

Complications

Testicular blood supply derives from the internal spermatic artery, cremasteric and vassal arteries that penetrate the tunica albuginea and septa (Ron-El *et al.*, 1998). Testicular damage secondary to a surgical noxa is either the consequence of the interference of the vascular supply to the seminiferous tubules or an increased intratesticular pressure secondary to bleeding enclosed within the tunica albuginea given its non-flexible characteristics (Silber, 2000). Less invasive techniques, such as FNA or MD aim to reduce the incidence of complications after sperm retrieval, however, no randomized study has compared the rate of short and long-term consequences of these surgical approaches.

Haematoma

The development of intratesticular haematoma has been observed in up to 80% of patients undergoing TESE with single or multiple biopsies based on ultrasounds performed 3 months after surgery (Schlegel and Su, 1997).

Several studies have documented a lower incidence of hemorrhagic complications following MD compared with the conventional technique (Table 2). A prospective study evaluating 100 patients observed a higher occurrence of hypoechoic focal lesions on ultrasound suggestive of haematoma at 1 and 3 months after TESE compared with MD (58.3 versus 15%; 51.7 versus 6.7%, respectively) (Amer *et al.*, 2000). In agreement with these findings, Okada *et al.*, (2002) observed a higher rate of hypoechoic areas (51 versus 12%) after conventional TESE compared with MD 1 month after surgery. Moreover, a large series of 83 TESE and 460 MD procedures showed a significantly higher frequency of acute findings at ultrasound performed 3 months after the surgery in TESE cases (Ramasamy *et al.*, 2005). Nevertheless, it should be acknowledged that MD requires special surgical skills along with the need of magnification equipment, thus making it less accessible to all centres.

Although multiple controlled observational studies have compared FNA with TESE only a few studies reported on complications (Table 3). Harrington *et al.* (1996) reported intratesticular bleeding in 29% of the cases after TESE and in 7% after FNA. Friedler *et al.* (1997) documented one case of bleeding after FNA and two cases in the TESE group. On the other hand, Khadra *et al.* (2003) reported no haemorrhagic complications for both FNA and TESE in a group of 84 men.

Fibrosis

Multiple studies have shown ultrasonographic changes after TESE, which have been attributed to the development of scar tissue (Schlegel and Su, 1997; Ron-El *et al.*, 1998; Amer *et al.*, 2000; Yagan, 2000). In a series of 14 NOA patients, hypoechogenic lesions and echogenic foci were observed in 64 and 54%, respectively, of the patients after 3 and 6 months of the biopsy (Ron-El *et al.*, 1998). In addition, a follow-up study of 6 months post-TESE showed that 9 out of 14 patients had parenchymal calcifications or linear hyperechoic focus suggestive of scar tissue (Schlegel and Su, 1997). Nonetheless, another study reported that only one of 30 patients showed pathological findings at ultrasound after a mean follow-up period of 18 months (Schill *et al.*, 2003).

Although TESE has been associated with a lesser trauma to the testis, Shufaro *et al.* (2002) found in an animal model (normal male rats) extensive architectural distortion of the tubules, chronic inflammation, necrosis and degenerative changes associated with the puncture itself, but not related to the inflicted negative suction. On the other hand, TESE showed only local chronic inflammation and degenerative cells on the biopsied area.

Only two studies compared the incidence of fibrosis at 6 months after TESE and MD. Amer *et al.* (2000) observed fibrosis in 30% of the cases of conventional TESE after 6 months compared with 3.3% on the MD group. Similar results were found on another study with focal echogenic lesions on 23% of the patients in the TESE group compared with none in the MD group (Okada *et al.*, 2002).

Nevertheless, MD has been associated as well with segmental devascularization in four patients whom underwent colour Doppler after 3 months of the procedure (Ramasamy *et al.*, 2005).

Testicular atrophy

Severely oligospermic men show significantly lower serum testosterone levels and higher LH and estradiol levels than fertile men derived from an impaired Leydig cell function (Andersson *et al.*, 2004). A decrease in Leydig cell function after testicular biopsy can further reduce serum testosterone levels because of vascular damage with serious long-term consequences such as osteoporosis, increased insulin resistance and depression (MacIndoe, 2003). Therefore, NOA men should be considered to be in high risk of developing androgen deficiency and hypogonadism after TESE (Tash and Schlegel, 2001; Bouloux *et al.*, 2002; Schill *et al.*, 2003).

The removal of a lesser amount of testicular tissue with MD compared with conventional TESE could reduce the risk of these complications (Okada *et al.*, 2002). Two cases of clinical unilateral testicular atrophy were documented and confirmed by colour Doppler ultrasound in a follow-up study of 64 patients after TESE (Schlegel and Su, 1997). Moreover, a reduction in testicular volume of >2 ml has been reported to be higher after conventional TESE than MD (Okada *et al.*, 2002). Nonetheless, a significant decrease on testosterone levels has also been shown following MD (Ramasamy *et al.*, 2005; Everaert *et al.*, 2006). Ramasamy *et al.* (2005) found no difference between conventional TESE and MD on the percentage of patients in whom testosterone levels returned to pre-surgical values after 12 months follow-up (85% TESE versus 95% MD). Furthermore, no significant difference was reported between these techniques on serum total and free testosterone concentrations after 1, 6 and 12 months of the surgery (Komori *et al.*, 2004).

Live birth rate

ICSI cycles with testicular spermatozoa are less successful in NOA compared with OA (Vernaev *et al.*, 2003; Nicopoullos *et al.*, 2004). A meta-analysis showed a significantly lower implantation rate when frozen-thawed sperm had been used compared with fresh sperm (relative risk, RR 1.75; 95% CI: 1.10–2.80), however, no differences were observed in fertilization and ongoing pregnancy rates (Nicolopoulos *et al.*, 2004).

Table 4: Summary of studies comparing the clinical outcome of ICSI cycles with TESE and FNA

| Study | Outcome measure | TESE (%) | FNA (%) |
|-----------------------------|----------------------------|----------|---------|
| Mercan <i>et al.</i> (2000) | Implantation rate/embryo | 13.3 | 20.7 |
| | Clinical pregnancy rate/ET | 28.9 | 46.0 |
| Nassar <i>et al.</i> (2001) | Clinical pregnancy rate/ET | 17.6 | 20.0 |
| | Delivery rate/cycle | 11.5 | 8.2 |
| Khadra <i>et al.</i> (2003) | Delivery rate/ET | 17.6 | 11.4 |
| | Clinical pregnancy rate/ET | 39.1 | 49.8 |

ET, embryo transfer; FNA, fine needle aspiration; TESE, testicular sperm extraction.

Only a few studies are available concerning the influence of the retrieval technique used on the chance of success (Table 4). It has been suggested that testicular spermatozoa retrieved through FNA results in higher implantation and pregnancy rates compared with TESE because of a lesser degree of spermatogenetic impairment in these patients (Mercan *et al.*, 2000). Nonetheless, the former was only shown in an observational study that reported on the outcome of 291 ICSI cycles in NOA men (20.7 versus 13.3% implantation rate per embryo and 46 versus 28.9% clinical pregnancy rate per embryo transfer) (Mercan *et al.*, 2000). An important limitation of this study is that the histological diagnosis was available only for 50 of 63 patients in the FNA group and 129 of 228 in the TESE group. Khadra *et al.* (2003) described a higher fertilization rate with FNA compared with TESE, however, no difference was observed on the cleavage and pregnancy rates. Only one study compared the delivery rates per cycle of FNA and TESE showing no significant difference (8.2% for FNA versus 11.5% for TESE) (Nassar *et al.*, 2001). In addition, no differences were observed on the fertilization, implantation and clinical pregnancy rates. Although this study showed that both groups were comparable for age, histopathology, number of oocytes retrieved and number and quality of embryos transferred, the limited number of patients analysed (60 patients, 75 cycles) does not enable to draw definitive conclusions.

No randomized or observational study has so far compared live birth or clinical pregnancy rates between cycles using spermatozoa retrieved through MD and conventional TESE.

Conclusions

Current guidelines on surgical sperm retrieval techniques for NOA are only based on observational studies; hence no definitive conclusions can be drawn. In addition, most of these studies lack adequate control groups and used different criteria to include patients. However, it can be concluded that according to the best available evidence, TESE in NOA men should be performed taking multiple samples because of the superior chance of retrieving spermatozoa compared with single biopsy. On the other hand, TESE with multiple biopsies results in a higher SRR than FNA especially in cases of SCO and MA. MD seems to perform better than conventional TESE only in cases of SCO where tubules containing active focus of spermatogenesis can be identified. Although MD reduces the amount of tissue required to retrieve sperm, this technique is not exempt of complications such as fibrosis and

hypogonadism. Ultimately, the only way to establish if optical magnification offers a significant benefit on SRR will be through a large multicentre randomized study.

MD appears to be the safest technique regarding post-operative complications followed by FNA. Nonetheless, recent evidence on animal models raises concerns on the long-term consequences of FNA because of a higher disturbance of the tubular architecture compared with open biopsy. Moreover, the fact that conventional TESE performed by a skilled surgeon achieves high rates of sperm retrieval even after two or three repeated biopsies reinforces this strategy as a safe procedure.

Only a few small studies could be identified concerning the influence of the sperm retrieval technique on the pregnancy and live birth rate, hence, no definitive conclusions can be made. It is possible that the enhanced outcome reported for FNA results from selection bias since sperm retrieval through this technique is mostly achieved in milder cases. Thus, so far it appears to be no impact of the technique itself on the success rate of the cycle.

It is mandatory that future studies in NOA report histopathology using a comparable system such as the recently proposed by McLachlan *et al.* (2007). The former avoids the use of confusing terms, which could partially explain the differences on reported sperm recovery rates in NOA patients.

Further research should focus on the added value of non-invasive techniques such as Doppler ultrasound on the identification of the most likely areas from which to find sperm.

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