Surgical sperm recovery for intracytoplasmic sperm injection: which method is to be preferred?

Herman Tournaye

Centre for Reproductive Medicine, Dutch-speaking Brussels Free University, Laarbeeklaan 101, B-1090 Brussels, Belgium: e-mail: tournaye@usa.net

Different methods for recovering epididymal or testicular spermatozoa have been described and each has its drawbacks and advantages. Percutaneous aspiration of the testis may be the method of choice in cases of irreparable obstructive azoospermia. Using a 21-gauge needle, spermatozoa may be recovered in 96% of patients. More patients undergoing fine-needle aspiration experienced less pain than expected as compared with those undergoing open biopsy. Microsurgical epididymal sperm aspiration (MESA) is the preferred method in patients with an incomplete work-up because, if indicated, a vasoepididymostomy can be performed concomitantly with a full scrotal exploration. In azoospermic patients with testicular failure, the sperm recovery rate, i.e. the chance of finding at least one spermatozoon, is around 50% after multiple open biopsies. However, the fertilization rates after intracytoplasmic sperm injection (ICSI) are significantly lower than in men with normal spermatogenesis, and complete fertilization failure may occur more frequently. Although the combination of testicular sperm extraction (TESE) and ICSI may be the sole treatment available for infertility because of non-obstructive azoospermia, the overall success rate is limited and ongoing pregnancies are obtained in ≤20% of ICSI cycles. In patients with incomplete Sertoli cell-only syndrome, testicular damage may be limited by use of a selective microsurgical approach; less invasive methods such as fine-needle aspiration are not useful in these patients. Of 14 patients with primary testicular failure as proven by histopathology, only in one case (7.1%) were spermatozoa recovered by multiple aspirations, while in nine cases (64.3%) spermatozoa were recovered by open biopsy. Although the pregnancy rates reported after ICSI with frozen–thawed testicular spermatozoa from patients with primary testicular failure are relatively low, the recovery of testicular spermatozoa by open biopsy followed by cryopreservation may be the method of choice by which to prevent repeat surgery and pointless ovarian stimulation in the female partner.

Key words: azoospermia/epididymis/FNA/ICSI/MESA/TESE/testis
H.Tournaye

Introduction

In recent years the role of assisted reproductive technology has become more important in the treatment of male infertility. For many infertile men, such technology represents the only way for them to father children. This is especially true for azoospermic men with congenital absence of the vas deferens (CBAVD) or primary testicular failure. The first pregnancies with epididymal spermatozoa were achieved with conventional in-vitro fertilization (IVF) (Temple-Smith et al., 1985; Silber et al., 1987). Although fertilization in vitro has been reported, no pregnancies have ever been reported after conventional IVF with testicular spermatozoa (Hirsh et al., 1993a). It was only with the introduction of intracytoplasmic sperm injection (ICSI) that high fertilization rates were obtained with epididymal spermatozoa (Tournaye et al., 1994) and that the first pregnancies were achieved with testicular spermatozoa (Craft et al., 1993; Schoysman et al., 1993). In recent years, pregnancies have even been reported after ICSI with round spermatids recovered either from the ejaculate (Tesarik et al., 1995) or from a testis biopsy (Antinori et al., 1997). These initial successes with surgically recovered spermatozoa were followed by an explosion of alternative methods for recovering spermatozoa. Their respective acronyms are as follows: MESA, microsurgical epididymal sperm aspiration; PESA, percutaneous epididymal sperm aspiration; TESE, testicular sperm extraction (refers to open excisional testicular biopsy); TESA, testicular sperm aspiration (refers to any method by which testicular spermatozoa are aspirated percutaneously); FNA, fine-needle aspiration (refers to any aspiration technique using needles of 21-gauge or thinner). It is clear that even more alternative techniques and acronyms will follow. Each technique, however, has its drawbacks and advantages and the question may therefore arise as to which technique is preferable for obtaining spermatozoa for ICSI when no other treatment option is available to cure the problem of azoospermia.

Microsurgical epididymal sperm aspiration

The MESA technique that we have adopted has been described in detail previously (Tournaye et al., 1997b). The main drawback of MESA is that it is an invasive and expensive procedure requiring a basic knowledge of epididymal anatomy and of microsurgical techniques.

Scrototomy is usually performed under general or loco-regional (cord block) anaesthesia. An operating microscope with ×50–80 magnification is preferable, although binocular loupes may also be effective. When performed by a skilled surgeon with a proper microsurgical approach, the procedure causes minimal fibrosis and has minimal risk of creating postoperative obstructions of the epididymal tubule. The major benefit of this procedure is its diagnostic power: a full scrotal exploration can be performed, and whenever indicated, a vasoepididymostomy can be performed concomitantly. Furthermore, the number of
Preferred method for surgical sperm recovery

spermatozoa retrieved is high, which facilitates cryopreservation (Devroey et al., 1995; Oates et al., 1996; Holden et al., 1997). So far, there are no published data comparing ICSI using fresh or frozen–thawed spermatozoa. When we performed a retrospective analysis of our consecutive data on ICSI with epididymal spermatozoa, we found no differences in terms of fertilization and clinical pregnancy rates for ICSI with either fresh or frozen–thawed epididymal spermatozoa (Tournaye et al., 1999). The two-pronuclear (2PN) fertilization rates per metaphase-II oocyte injected were 59.4 and 56.3% respectively and the ongoing pregnancy rates were 26.7 and 21.2% respectively per cycle.

Recovery of epididymal spermatozoa during a diagnostic procedure is certainly a valid option. ICSI may be performed later, and even in another centre, using the frozen–thawed epididymal spermatozoa, without jeopardizing the ICSI success rate.

Percutaneous epididymal sperm aspiration

This technique is a less invasive variant of MESA. By means of a percutaneous puncture using a 19-gauge needle, epididymal spermatozoa may be aspirated blindly from the epididymis under local or loco-regional anaesthesia. This technique has been described in detail by Craft et al. (1995).

Its non-invasive character is the most important advantage of PESA. It can be easily performed on an outpatient basis, and the technique is quick and simple when compared to MESA and is certainly more cost-effective. It has been argued that there may be fewer complications after PESA than after MESA (Tsirogotis et al., 1995). However, there are almost no published data comparing PESA with MESA in terms of outcome, complications or patient comfort. In a small population of 20 vasectomized men, the sperm recovery rate (i.e. the chance of finding at least one spermatozoon) reported after PESA was comparable to that after MESA (Collins et al., 1996).

In a series of 181 PESA-ICSI cycles reported by Meniru (1998), epididymal spermatozoa were recovered in 151 cycles (83%). In two cases out of the 30 failures, spermatozoa were obtained by MESA, and in 19 cases spermatozoa were found after a testicular sperm recovery procedure. The nine remaining patients turned out to have primary testicular failure. In 151 cases, ICSI was performed with fresh spermatozoa obtained after PESA. The fertilization rate was 54.7% and the ongoing pregnancy rate 33.4% per ICSI cycle.

Although it has been occasionally mentioned that epididymal spermatozoa may also be cryopreserved after PESA, no published data could be found to support this approach. It has also been stated that PESA may be repeated in the same patient several times with good results.

The main criticism of the PESA technique is that blind percutaneous puncture may cause inadvertent damage to the fine epididymal structures and produce uncontrolled bleeding, causing postinterventional fibrosis (Girardi and Schlegel,
Figure 1. Average VAS (visual analogue scale) scores of stress and pain experienced by patients undergoing either fine-needle aspiration (FNA) or open excisional biopsy (open) of the testis at different moments during their treatment. VAS scores were taken when patients were waiting to undergo their surgery (waiting), just before the surgery (pre-) and after the surgery (post-).

1996). Another disadvantage is the impossibility of performing a proper diagnostic work-up and concomitant reconstructive microsurgery.

Testicular sperm aspiration

Several methods have been described for percutaneous needle aspiration of the testis to obtain a histological or cytological diagnosis of spermatogenesis or to recover spermatozoa: biopsy gun (Morey et al., 1993; Hovatta et al., 1995), or 19- or 21-gauge needle (Bourne et al., 1995; Craft et al., 1997; Tournaye et al., 1998). When 21-gauge or finer needles are used (fine-needle aspiration or FNA), aspiration may be performed without any anaesthesia, while the biopsy gun and 19-gauge needles require local or loco-regional anaesthesia. The aspiration technique is simple and quick and is non-invasive except for biopsy gun systems or analogues.

In patients with normal spermatogenesis, a sperm recovery rate of 96% may be obtained by 21-gauge FNA (Tournaye et al., 1998). However, 21-gauge FNA may provide material suitable only for cytological assessment, while using larger needles may provide tissue cylinders which allow an accurate histopathological examination (Mallidis and Baker, 1994; Craft et al., 1997).

We have performed a retrospective controlled comparison of open biopsy (TESE) and FNA and found no differences in fertilization rates, cleavage rates or implantation rates after ICSI (Tournaye et al., 1998).

The aspiration procedure is assumed to be more patient-friendly because of its minimally invasive character. In a prospective stress and pain assessment using a visual-analogue scale method (see Godoy et al., 1993 for description, we tried to assess stress and pain as experienced by patients (n = 54) undergoing either open biopsy under local anaesthesia (n = 22) or FNA under local anaesthesia (n = 32). The average stress and pain scores were higher in patients undergoing aspiration (see Figure 1). However, when these data were expressed
as proportions of patients experiencing more or less pain than anticipated, FNA had more patients experiencing less pain than expected compared with open biopsy (Figure 2). Some patients were indeed very anxious about the needle, which may explain the higher average stress score in FNA patients. Furthermore, a few patients reported the FNA procedure as extremely painful, which increased the average pain score in the FNA group but did not increase the proportion of patients undergoing FNA and experiencing more pain than anticipated.

**Testicular sperm recovery by excisional biopsy**

The retrieval of testicular spermatozoa by open excisional biopsy is equivalent to a diagnostic testicular biopsy procedure. In order to recover testicular spermatozoa, the sampled tissue is disrupted and minced or digested by enzymes in order to release the spermatozoa from the seminiferous tubules. This technique of testicular sperm extraction (TESE) is even simpler than PESA and may be performed under local anaesthesia. In patients with normal spermatogenesis, TESE gives a 100% recovery rate (Tournaye et al., 1996a, 1997a). In patients with testicular failure showing different degrees of maturation arrest, germ-cell aplasia (Sertoli cell-only patterns) recovery rates are ~50% (Figure 3). In patients with 47,XXY Klinefelter syndrome, spermatozoa may be recovered from about half of the patients (Tournaye et al., 1996b, 1997b). When spermatozoa are found, ICSI may be performed and pregnancies obtained. Pregnancies have even been obtained for patients with 47,XXY Klinefelter syndrome, and the first babies born were reported recently (Tournaye et al., 1997b). However, as reported earlier, the fertilization rate after ICSI with spermatozoa from patients with testicular failure is significantly lower (Tournaye et al., 1996a). This is confirmed in our larger consecutive series, which is given in Table I (extension of previously
Table I. Results of 485 intracytoplasmic sperm injection (ICSI) procedures according to histological category using testicular spermatozoa from patients with azoospermia.

<table>
<thead>
<tr>
<th>Category</th>
<th>Normal</th>
<th>Aplasia</th>
<th>Arrest</th>
<th>Hypoplasia</th>
<th>Sclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ICSI procedures</td>
<td>310</td>
<td>81</td>
<td>49</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>No. of mature oocytes injected (A)</td>
<td>3632</td>
<td>1039</td>
<td>524</td>
<td>338</td>
<td>146</td>
</tr>
<tr>
<td>No. of oocytes successfully injected (B)</td>
<td>3329 (92)</td>
<td>918 (88)</td>
<td>490 (93)</td>
<td>315 (93)</td>
<td>145 (99)</td>
</tr>
<tr>
<td>No. of oocytes showing 2PN (C)</td>
<td>2205 (66)</td>
<td>488 (53)</td>
<td>221 (45)</td>
<td>193 (61)</td>
<td>73 (50)</td>
</tr>
<tr>
<td>No. (%) cycles with 100% fertilization failure</td>
<td>12 (4)</td>
<td>3 (4)</td>
<td>6 (12)</td>
<td>0</td>
<td>2 (12)</td>
</tr>
<tr>
<td>No. of good-quality embryos</td>
<td>1576 (71)</td>
<td>355 (73)</td>
<td>168 (76)</td>
<td>151 (78)</td>
<td>59 (81)</td>
</tr>
<tr>
<td>No. of embryo transfers</td>
<td>288 (93)</td>
<td>76 (94)</td>
<td>38 (77)</td>
<td>25 (86)</td>
<td>14 (87)</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>104 (33.5)</td>
<td>16 (19.8)</td>
<td>9 (18.4)</td>
<td>11 (37.9)</td>
<td>3 (18.5)</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>12.1</td>
<td>9.1</td>
<td>6.7</td>
<td>15.9</td>
<td>5.3</td>
</tr>
</tbody>
</table>

---

a Extension of previously reported data (Tournaye et al., 1996a, 1997b).
bPercentage of A is given in parentheses.
cPercentage of B is given in parentheses.
dPercentage of C is given in parentheses.
eTransfer rate is given in parentheses.
fPregnancy rate/ICSI cycle is given in parentheses (pregnancy was determined by raised human chorionic gonadotrophin concentration).
gImplantation rate defined as positive heartbeat/embryo transferred.

Reported data in Tournaye et al., 1996a, 1997b). Furthermore, in these patients the complete fertilization failure rates may be higher: in up to 12% of cycles, no fertilization will ensue. In particular, implantation rates are significantly decreased in patients with non-obstructive azoospermia. Although testicular sperm recovery represents a major breakthrough for patients suffering from non-obstructive azoospermia, the outcome after ICSI remains poor.

In many patients, testicular spermatozoa may be recovered only by excising multiple biopsies (Tournaye et al., 1995, 1996b), which may cause localized
Preferred method for surgical sperm recovery

testicular fibrosis (Schlegel and Su, 1997). Casuistic reports of less invasive methods by which to retrieve spermatozoa from patients with non-obstructive azoospermia have been published (Lewin et al., 1996). Although for diagnostic purposes multiple needle aspiration was reported to give reliable results (Turek et al., 1997), the technique may have its limitations for recovering testicular spermatozoa in patients with testicular failure. Testicular spermatozoa were obtained by open excisional biopsy in 13 out of 32 (40.6%) patients with primary testicular failure as proven by histopathology, while with multiple FNA using a 21-gauge needle spermatozoa were found in only four (12.5%) cases (Friedler et al., 1997). These findings corroborate our own experience in a prospective controlled study: out of 14 patients with primary testicular failure as proven by histopathology, in only one (7.1%) case were spermatozoa recovered by multiple FNA using a 21-gauge needle. However, with the open biopsy approach, we were able to recover spermatozoa in nine out of the 14 cases (64.3%). In patients with non-obstructive azoospermia, it may therefore be preferable to perform open biopsies in order to recover testicular spermatozoa for ICSI, or to use thicker aspiration needles.

In order to minimize tissue damage when taking multiple excisional biopsies, small tissue samples may be taken using a microsurgical approach as proposed by Schlegel (1998). After opening the tunica albuginea, the seminiferous tubules are exposed at ×40–80 magnification and the more distended tubules may be selected for micro-excision. In our preliminary experience, this technique may be beneficial in patients with incomplete Sertoli cell-only syndrome, where there is a substantial difference in diameter of empty and filled tubules. However, in patients with incomplete maturation arrest, we have not been successful in using this selective microsurgical approach because of the lack of any difference in tubule diameter.

A major advantage of the open approach is the possibility of freezing testicular tissue and of performing ICSI whenever the final histopathology shows spermatozoa, thus preventing pointless ovarian stimulation in the female partner (Salzbrunn et al., 1996). However, in some patients, ICSI may not be proposed because the final histopathology may not show testicular spermatozoa or late spermatids. Although histopathology is the best indicator of whether spermatozoa for ICSI may be present or not (Tournaye et al., 1997a), in patients with maturation arrest the predictive power is insufficient to exclude a patient from treatment based on a negative biopsy result (Mulhall et al., 1997; Tournaye et al., 1997a). In any case, cryopreservation of testicular spermatozoa may prevent repeat surgery when pregnancy does not occur after ICSI. So far there have been several more or less casuistic reports on this approach (Fischer et al., 1996; Gil-Salom et al., 1996; Hovatta et al., 1996; Podsiadly et al., 1996; Romero et al., 1996; Khalifeh et al., 1997).

The largest series is that reported by the Hamburg group, which included 246 patients undergoing 406 ICSI cycles using frozen–thawed testicular spermatozoa in the approach described by Salzbrunn (1996). The 2PN fertilization rate per successfully injected metaphase-II oocyte was 48.3%, and 60.1% of 2PN oocytes
became transferable embryos. The overall pregnancy rate was 18.5% per ICSI cycle and the ongoing pregnancy rate was 16% per ICSI cycle. The implantation rate per embryo was 9% (Fischer et al., 1998). The pregnancy rate reported in this series, which consisted of patients with both obstructive and non-obstructive azoospermia, was rather low.

Nevertheless, considering the low pregnancy rates after ICSI with fresh testicular spermatozoa from patients with primary testicular failure (see Table I), the use of frozen–thawed testicular spermatozoa may be preferable to repeat surgery in these patients.

**Other methods by which to retrieve spermatozoa surgically**

There have been reports on alternative methods by which to recover spermatozoa from the male genital tract, e.g. from the vas deferens for conventional IVF (Pryor et al., 1984; Hirsh et al., 1993b; Utsunomiya et al., 1997). These techniques are reported to yield many spermatozoa and they may be especially useful in patients with anejaculation in whom vibrostimulation or electroejaculation has failed. However, both vasal sperm retrieval and retrograde epididymal sperm collection may lead to the recovery of more dysfunctional spermatozoa or spermatozoa with DNA damage, since it is known that, in patients with obstruction, the most distal spermatozoa are the most senescent (Silber, 1989).

**Summary**

Since the introduction of ICSI, both epididymal and testicular spermatozoa have been used successfully to obtain pregnancies. Different methods for recovering epididymal or testicular spermatozoa have been described and each has its drawbacks and advantages. The question may therefore arise as to which method is preferable. From data in the literature and from our own experience, it appears that percutaneous aspiration of the testis may be the method of choice in patients with normal spermatogenesis, since the sperm recovery rate is high and patients experience less pain than those undergoing an open biopsy. In patients with obstructive azoospermia who have not had a work-up or have had an incomplete one, MESA is the preferred method because a full scrotal exploration can be performed and, whenever indicated, a vasoepididymostomy may be performed concomitantly.

In azoospermic patients showing different degrees of maturation arrest or germ-cell aplasia, recovery rates are ~50% after multiple open biopsies. Because the fertilization rates after ICSI are significantly lower and because of high fertilization failure rates, ongoing pregnancies are obtained in only 10% of ICSI cycles. The surgical recovery of testicular spermatozoa by (multiple) open biopsies with cryopreservation, followed by thawing only after histopathology demonstrates the presence of spermatozoa, may be the method of choice by
which to prevent repeat surgery and pointless ovarian stimulation in the female partner.

References


Levin, A., Weiss, D.B., Friedler, S. et al. (1996) Delivery following intracytoplasmic injection of
H. Tournaye

mature sperm cells recovered by testicular fine needle aspiration in a case of hypergonadotropic azoospermia due to maturation arrest. Hum. Reprod., 11, 769–771.


Preferred method for surgical sperm recovery


