Fine needle aspiration versus open biopsy for testicular sperm recovery: a controlled study in azoospermic patients with normal spermatogenesis

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This retrospective controlled study aimed at comparing two techniques for recovering testicular spermatozoa in azoospermic patients undergoing intracytoplasmic sperm injection (ICSI). 102 men suffering from infertility because of obstructive azoosperma had ICSI using testicular spermatozoa recovered either by open excisional biopsy (n = 51), or by fine needle aspiration (FNA) (n = 51). A higher average number of spermatozoa were recovered after open biopsy than after FNA, but no significant differences in either fertilization rates or cleavage rates were observed after ICSI with spermatozoa recovered by the two techniques. Neither was there any significant difference in ongoing pregnancy and implantation rates: in the FNA group, these figures were respectively 19.6% per cycle and 7.8% per embryo transferred and in the open biopsy group 21.6 and 7.1%. We conclude that ICSI with testicular spermatozoa recovered by an open excisional approach. The present study therefore aimed at comparing the results of ICSI using testicular spermatozoa recovered by an open excisional technique and ICSI using testicular spermatozoa recovered by a percutaneous fine needle aspiration (FNA) technique.

Materials and methods

All couples included in this study were suffering from long-standing infertility because of surgically irreparable obstructive azoosperma. They were undergoing treatment by ICSI with testicular spermatozoa. They were informed about the current concerns (Cummins, 1997; Tournaye and Van Steirteghem, 1997) regarding this technique and the possible complications of testicular sperm recovery procedures. All male partners were examined and their testicular volumes were measured with a Prader orchidometer. Their further work-up also included serum follicle stimulating hormone (FSH) assessment, peripheral blood karyotype and screening to see if they were carriers of cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations whenever indicated. All were reported to have normal spermatogenesis at previous testicular biopsies performed by the referring physician. Testicular spermatozoa were recovered either by FNA using a 21-gauge butterfly needle or by an open excisional procedure. All testicular sperm recovery procedures were scheduled on the day of ovum retrieval in the wife. The work-up of the female partner and the methods of controlled-ovarian stimulation, ultrasound-guided oocyte retrieval and luteal phase support have been described previously (Tournaye et al., 1992). For testicular sperm recovery, the scrotum was disinfected with chlorhexidine digluconate (HAC; Zeneca, Ghent, Belgium) and unilateral local anaesthesia with 1% mepivacaine (Scandicaine; Astra Pharmaceuticals, Brussels, Belgium) was performed on the hemiscrotum containing the larger testis.

Fine needle aspiration procedure

The tubing of the 21-gauge butterfly needle (Terumo infusion set, SV-21 BL; Terumo, Brussels, Belgium) was filled with ~100 µl of HEPES-buffered Earle’s medium as used for sperm preparation, using
a 20 ml syringe. With a single puncture the needle was inserted into the testicular mass and care was taken not to move the needle once this was inserted. After repeated (5–10 times) aspiration movements the tubing was clamped, the syringe released and the needle was withdrawn. The contents of the needle and the tubing were flushed into 14.5 µl micro-droplets of Earle’s medium under liquid paraffin oil in a Petri dish (Falcon Plastics, no. 1006; Becton-Dickinson, Aalst, Belgium) so as to divide and dilute the volume over different droplets. In the adjacent laboratory, these droplets were then checked at ×400 magnification under an inverted microscope for the presence of spermatozoa. The number of testicular spermatozoa was scored subjectively: when spermatozoa were immediately observed, the sample was rated 3+, when spermatozoa were observed only after an extensive search, the sample was rated 1+. Any situation in between these was rated 2+. When spermatozoa were observed, the Petri dish was brought to the ICSI laboratory. For ICSI, the spermatozoa were retrieved from the first Petri dish and pooled into a central droplet of a second Petri dish prepared for micro-injection.

Open testicular biopsy procedure
A 0.5–1 cm incision was made through the skin and the underlying layers. After incision of the tunica albuginea, gentle pressure was applied to the testicular mass and a small specimen (maximum 0.05 ml) of the testicular mass which protruded was removed by a pair of curved scissors. This specimen was rinsed in HEPES-buffered Earle’s medium containing 0.4% human serum albumin (Belgian Red Cross, Brussels, Belgium) and then transferred to a second Petri dish containing the same medium. In the adjacent laboratory this tissue was shredded using glass microscope-cover slides as described elsewhere (Verheyen et al., 1995). The preparation was checked for the presence of spermatozoa by using an inverted microscope at ×400 magnification. Here too, a subjective scoring of the number of spermatozoa recovered was made using the same scale as described above.

ICSI, assessment of fertilization and embryo cleavage
In the ICSI laboratory, the numbers of spermatozoa recovered were calculated from the suspensions. A 5 µl aliquot of the supernatant was microscopically assessed and the absolute number of spermatozoa present in the whole suspension was extrapolated from the number of spermatozoa in this aliquot. The protocol for micro-injection has been described in full detail elsewhere (Van Steirteghem et al., 1995). Eighteen hours after ICSI, oocytes were checked for the presence of two pronuclei under an inverted microscope at ×400 magnification. The embryo cleavage of the pronuclear stage oocytes was evaluated after another 24 h of in-vitro culture. Embryos with <50% of their volume filled with anucleate fragments were considered transferable. On day 2 after sperm recovery and ICSI, as a rule up to three cleaving embryos were replaced into the uterine cavity of patients under 40 years of age. Pregnancy was confirmed by the measurement of increasing concentrations of serum human chorionic gonadotrophin (HCG) on at least two occasions from day 10 after embryo transfer. The observation of a gestational sac by echographic screening at 7 weeks of pregnancy was indicative of a clinical pregnancy. Ongoing pregnancy was defined as a gestation evolving beyond 20 weeks. Pregnant patients were asked to co-operate in our prospective follow-up study of pregnancies after ICSI (Bonduelle et al., 1996; Wisanto et al., 1996).

Statistical analysis
The choice of the method for testicular sperm recovery was made by the patients. Patients who underwent successful fine needle aspiration for testicular sperm recovery were matched retrospectively with patients who underwent a successful open excisional biopsy in the same study period. They were matched for rank of trial, female age and indication for testicular sperm recovery, but they were blinded for all other parameters before further analysis. Outcome measures are expressed as means with their 95% confidence intervals (CI). Whenever indicated, the χ² test was applied at the 5% level of significance using a statistical software package (Medcalc, Medcalc, Ghent, Belgium).

Results
Fifty-three men underwent fine needle aspiration (FNA) to recover testicular spermatozoa for ICSI. In two of these men (3.7%), an open biopsy was performed because the number of spermatozoa recovered by FNA was judged inadequate. Both had adequate numbers of spermatozoa after open biopsy. The 51 ICSI cycles using testicular spermatozoa retrieved by FNA (n = 51 patients) were retrospectively matched to 51 ICSI cycles with spermatozoa obtained after open excisional testicular biopsy (n = 51 patients). The rank of trial ranged from one to four with an average rank of trial of 1.7 ± 0.7 [95% confidence interval (CI) 1.4–1.8] for both groups. The mean age of the male patients undergoing FNA was 41.1 years (range 27–74 years, 95% CI 38.5–42.5 years). In open biopsy cycles the mean age (in years) was 39.0 (range 25–56 years, 95% CI 37.3–41.5 years). The ages of the female partners were respectively 34.2 ± 6.1 years, 95% CI 32.8–35.5 years) and 34.0 ± 4.9 (range 25–44 years and 95% CI 32.7–35.4 years) for FNA and open biopsy respectively. The duration of infertility ranged from 1 to 20 years in the FNA group (average 7.3 ± 5.5 years, 95% CI 5.8–9.4 years) and in the open-biopsy group from 2 to 20 years (6.7 ± 4.1 years, 95% CI 5.1–8.3 years). No significant differences were observed in testicular volumes between patients undergoing FNA and those undergoing open biopsy: the volumes for the right testes were 24.5 ± 7.0 ml (95% CI 22.6–26.4) versus 21.9 ± 5.7 ml (95% CI 19.3–24.8) and 23.6 ± 6.7 ml (95% CI of the mean 21.3–25.7) versus 21.7 ± 6.1 ml (95% CI of the mean 19.1–24.2) respectively for the left testes.

Table I shows the results for testicular sperm recovery. The average numbers of punctures or excisions needed to recover spermatozoa were not significantly different. However, significantly more spermatozoa were recovered in the open-biopsy

<table>
<thead>
<tr>
<th>Procedures</th>
<th>Fine needle aspiration</th>
<th>Open biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (95% CI)</td>
<td>1.5 (1.3–1.7)</td>
<td>1.5 (1.3–1.8)</td>
</tr>
<tr>
<td>Range</td>
<td>1–3</td>
<td>1–4</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>1.8 (1.6–2.1)</td>
<td>2.5 (2.4–2.7)</td>
</tr>
<tr>
<td>Range</td>
<td>0.0022 (0.0006–0.0049)</td>
<td>0.1343 (0.0720–0.1855)</td>
</tr>
</tbody>
</table>
| CI = confidence interval. aFor FNA: number of punctures, for open biopsy: number of specimens excised.
| b95% confidence intervals for the mean.
| cScored on a scale from 1 to 3. |
biopsy–ICSI cycles (n = 14 pregnancies) and 27.5% for excisional biopsy (n = 14 pregnancies). In the FNA group, there were 13 embryonic sacs containing 12 viable fetuses (implantation rate of 7.1% per embryo transferred) and 11 pregnancies were ongoing.

**Discussion**

In all azoospermic patients with normal spermatogenesis, i.e. patients with so-called obstructive azoospermia, testicular spermatozoa may be recovered by open excisional biopsy (Tournaye et al., 1996, 1997). The open excisional biopsy technique is an invasive procedure which may cause discomfort for the patient, even if only taken through a small incision and even after meticulous haemostasis. Percutaneous puncture of the testis using a fine 21-gauge needle is a less invasive procedure which has been used successfully to recover testicular samples for diagnostic purposes. Yet this sampling method allows only cytological investigation because it contains only a limited quantity of aspirated testicular cells which may, however, be sufficient in order to establish the diagnosis of normal spermatogenesis (Foresta et al., 1992). A more invasive fine needle tissue aspiration technique has been reported to recover testicular spermatozoa for ICSI, and pregnancies have been obtained (Bourne et al., 1995). However, many patients may suffer varying degrees of discomfort during these tissue aspiration procedures, in which a 19-gauge biopsy gun-type needle is used. Others have successfully used a thinner 22-gauge needle (Lewin et al., 1996).

In the present study, we used a fine 21-gauge butterfly needle for testicular aspiration. Although patient comfort was not assessed in the present retrospective study, in our experience patients undergoing percutaneous sperm aspiration tended to report less pain and discomfort once at home than those who had had an open biopsy.

The percutaneous sperm aspiration, however, risks recovering only a few spermatozoa. In the present study, we found that although the number of samples taken, i.e. aspirates or biopsies, was comparable, significantly fewer spermatozoa were harvested after FNA than after open biopsy. In 51 out of 53 FNAs (96%), a sufficient number of spermatozoa were recovered to allow ICSI, and an open biopsy had to be performed in only two men. Once the oocytes were micro-injected, there was no further normally beyond 20 weeks of gestation. In the open biopsy group, there were 13 embryonic sacs containing 12 viable fetuses (implantation rate of 7.1% per embryo transferred) and 11 pregnancies were ongoing.

<table>
<thead>
<tr>
<th>Table II. Intracytoplasmic sperm injection (ICSI) with testicular spermatozoa recovered after fine needle aspiration (FNA) or open excisional biopsy in patients with normal spermatogenesis</th>
<th>Table III. Outcome of intracytoplasmic sperm injection (ICSI) procedures with testicular spermatozoa recovered after fine needle aspiration (FNA) or open excisional biopsy in patients with normal spermatogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICSI procedures</td>
<td>Fine needle aspiration</td>
</tr>
<tr>
<td>Number of OCC retrieved</td>
<td>691</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>13.5 (11.9–15.2)</td>
</tr>
<tr>
<td>Range</td>
<td>3–25</td>
</tr>
<tr>
<td>Number of oocytes injected</td>
<td>584</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>11.4 (9.8–13.0)</td>
</tr>
<tr>
<td>Range</td>
<td>0–25</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Cleavage rate</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>CI = confidence interval.</td>
<td>5</td>
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<tr>
<td>OCC: oocyte–cumulus complexes.</td>
<td>95% confidence interval of the mean.</td>
</tr>
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</table>

CI = confidence interval.  
95% confidence interval of the mean.  
Mean of percentages of injected oocytes showing 2 pronuclei per ICSI cycle.  
Mean of percentages of oocytes with 2 pronuclei developing into an embryo with <50% anucleate fragments per ICSI cycle.
difference in the 2 pronuclei (2-PN) fertilization rate or in the cleavage rate. The implantation rate, here defined as the number of fetuses with positive heart activity per embryo replaced, was not different between FNA and open biopsy.

From the results of this retrospective controlled study, we may conclude that FNA is an attractive alternative to excisional biopsy in azoospermic men with normal spermatogenesis. However, the present findings need to be confirmed in a prospective set-up. If confirmed, this simple aspiration procedure may become the standard technique for recovering testicular spermatozoa for ICSI in azoospermic men with normal spermatogenesis.

Although no large prospective data series are available on the long-term adverse effects of either technique, the less invasive percutaneous technique may cause less fibrosis than the open biopsy technique, especially when the aspiration is performed with a single puncture without further moving the needle as in the present study.

Ongoing pregnancies and births have been reported after ICSI with frozen–thawed testicular spermatozoa from excisional biopsies (Podsiadly et al., 1996; Romero et al., 1996). Thus testicular tissue recovered during a diagnostic procedure may be cryopreserved for later use. Some patients may therefore prefer to undergo a single more invasive open biopsy, with the possibility of freezing testicular tissue for later use and so omitting repeated aspirations. While theoretically tissue samples recovered after a more invasive testicular tissue aspiration technique may be frozen, the limited number of cells recovered by the less invasive fine needle aspiration technique does not easily allow cryopreservation. Since ICSI needs only a few spermatozoa in order to achieve fertilization, cryopreservation of testicular spermatozoa recovered after fine needle aspiration may become feasible in selected patients or after adaptations of the cryopreservation protocols, e.g. freezing of spermatozoa in empty zona pellucidae (Cohen et al., 1997).

Since after FNA fewer spermatozoa were harvested than after open biopsy, it is doubtful whether FNA using a 21-gauge needle may be an alternative to open biopsy in patients with non-obstructive azoospermia. Friedler et al. (1997) have already reported a lower sperm recovery rate after FNA (11%) than after open biopsy (43%) in 37 patients with non-obstructive azoospermia.

At present, candidate patients for treatment by ICSI with testicular spermatozoa need extensive counselling. They must be told about the benefits and drawbacks of each recovery technique. First, they must be informed about the adverse effects of the sampling technique. In a small case series, Schlegel and Su (1997) have described sonographic signs of testicular damage after multiple testicular biopsies. They must also be told about the concerns relating to the use of immature testicular spermatozoa from patients with normal spermatogenesis, i.e. concerns relating to genomic imprinting, even though at present these concerns may be of a merely academic nature (Tesarik and Mendoza, 1996).

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