Testicular biopty gun needle biopsy in collecting spermatozoa for intracytoplasmic injection, cryopreservation and histology

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Using testicular spermatozoa from either open biopsy (29 cycles) or biopty gun needle biopsy (49 cycles), a total of 81 intracytoplasmic sperm injection (ICSI) cycles among 57 couples were carried out from January, 1994 to September, 1997. In six cycles, no spermatozoa were obtained, and in three cycles spermatozoa from both needle and open biopsies were used. The fertilization (37% after open and 41% after needle biopsy) and pregnancy rates (29% per embryo transfer compared with 16% per embryo transfer) were similar after both open and needle biopsies. Five pregnancies were achieved among the 14 couples with non-obstructive azoospermia of the male partner, four of these after needle biopsy. It was possible to use cryopreserved testicular spermatozoa after both needle and open biopsies, and one pregnancy started after using cryopreserved testicular spermatozoa in both groups. Histological needle biopsy was carried out in 62 cases, and they were all diagnostic, giving 15–20 cross-sections of seminiferous tubuli per biopsy. Testicular needle biopsy using a 14 gauge biopsy needle gave a sufficient amount of tissue and spermatozoa for ICSI, cryopreservation and histology, even in non-obstructive azoospermia. This technique is simpler and cheaper than open biopsy and, hence, it can be regarded as the optimal method for the retrieval of testicular spermatozoa.

Key words: intracytoplasmic sperm injection/needle biopsy/pregnancy/testicular biopsy/testicular spermatozoa

Introduction

Testicular spermatozoa have been used in intracytoplasmic sperm injection (ICSI) in azoospermic men since the first successful pregnancies (Schoysman et al., 1993). Surgical open biopsy, often after failed attempts at retrieving spermatozoa by epididymal aspiration, has been used from the beginning (Devroey et al., 1994; Silber et al., 1995; Gil Salom et al., 1995; Abuzeid et al., 1997). It has also proved useful in non-obstructive azoospermia (Devroey et al., 1995; Kahraman et al., 1996). A simpler fine needle aspiration method was then adopted (Craft and Tsirigotis, 1995), and it has also been used successfully over the last few years (Bourne et al., 1995; Lewin et al., 1996; Friedler et al., 1997a). Testicular needle biopsy, which has been used to obtain biopsy samples for testicular histology (Rajfer and Binder, 1989; Morey et al., 1994), is yet another option that can be used to obtain testicular spermatozoa for ICSI (Hovatta et al., 1995). It has been shown to result in acceptable pregnancy rates in our first small group of patients treated this way.

Cryopreservation of testicular spermatozoa and testicular biopsy specimens is also feasible, resulting in pregnancies after ICSI (Gil-Salom et al., 1996; Hovatta et al., 1996; Podsiadly et al., 1996; Friedler et al., 1997b). Using cryopreservation, repeated invasive procedures can be avoided, but results connected with cryopreservation after needle aspiration or needle biopsy have not yet been reported.

In order to discover the optimal method to carry out testicular sperm retrieval, we analysed the results of our testicular sperm ICSI programme from January, 1994 to September, 1997. We analysed the clinical ICSI parameters and opportunities for cryopreservation, histological diagnosis, and practicality in an in-vitro fertilization (IVF) unit after both needle and surgical biopsies.

Materials and methods

Between January 1994 and September 1997, a total of 81 treatment cycles among 57 couples were carried out in the Infertility Clinic of the Family Federation of Finland, using testicular spermatozoa in ICSI. Of the 57 men, 43 had obstructive, and 14 non-obstructive azoospermia, 10 of whom were in the needle biopsy group. The mean age of the men was 35 years (range 22–47 years), and that of their female partners 32 years (range 22–43 years).

In the beginning of the programme (1994), the biopsies were taken in connection with scrotal exploration, if there was no earlier diagnostic biopsy. After 1995, if diagnosis had not been confirmed earlier by testicular biopsy, needle biopsy was carried out in the clinic prior to the ICSI cycle (nine men). Clinical andrological examinations were carried out in all cases, and the serum concentrations of gonadotrophins and testosterone were measured. A scrotal ultrasound scan was carried out if the testes did not appear normal on physical examination. From 1997, Y chromosome deletions and karyotypes were screened and genetic counselling was given when needed. Prenatal diagnosis was not routinely carried out, but it was available if the couples asked for it.

Four female partners had endometriosis, and ovulatory dysfunction was observed in four others.

Ovarian stimulation and oocyte retrieval

Ovarian stimulation was started by pituitary desensitization with intranasal buserelin (Suprecur; Hoechst, Frankfurt am Main, Germany), 1200 µg daily, from day 23 of the cycle. Stimulation with human menopausal gonadotrophin or follicle stimulating hormone
Embryos showing two pronuclei and two polar bodies were considered normally fertilized. Their quality was evaluated, and two-embryo transfers were carried out 24 h later. The remaining embryos of good quality were frozen, using propanediol as a cryoprotectant.

**Cryopreservation of testicular tissue**

For cryopreservation the testicular tissue was cut to small pieces in culture medium. Glycerol (Telko, Espoo, Finland) (7.4%) was used as a cryoprotectant. Glycerol was added slowly drop by drop to the medium containing the biopsy specimens. The biopsy pieces were frozen in 0.5 ml straws which were held for 30 min at –20°C and for 60 min in liquid nitrogen vapour before being placed into the liquid nitrogen.

**Statistical analyses**

Confidence intervals (95%) of means, the Shapiro–Wilks test, the two-sample *t*-test, the Mann–Whitney *U*-test and the χ² test were used as appropriate, using SPSS software (SPSS Inc, Chicago, IL, USA).

**Results**

Of the 81 treatment cycles, open surgical biopsy was carried out in 34 cycles, usually in connection with scrotal exploration. In 54 cycles, testicular tissue was obtained by needle biopsy. Both open and needle biopsy were carried out in seven cycles. This was done in the beginning of the programme, when the yields of spermatozoa using both methods in the same patients were compared. In six cycles (7% of the cycles), no spermatozoa were found (four patients having both open and needle biopsy, and two patients having open biopsy only). All the six patients had been scheduled for scrotal exploration and testicular biopsy without a previous histological analysis. They all had accepted donor spermatozoa as backup and it was used for fertilization of the oocytes. Later, ICSI using a needle biopsy was scheduled only if mature spermatids had been seen in a previous biopsy. Spermatozoa for ICSI were obtained in every such needle biopsy.

The mean serum FSH concentration of these six men was 13.6 (10.7–18.0) IU/L. Four had a histological diagnosis of spermatogenic arrest and two fibrosis.

The mean concentration of FSH among the 14 men with non-obstructive azoospermia was 12.4 (3.5–44.3) IU/L. It was 14.5 (3.5–44.3) in the needle biopsy group and 9.9 (4.0–18) in the open biopsy group. The histological diagnosis among these men were hypospermatogenesis in seven, partial spermatogenic arrest in five and fibrosis of testes in two cases. The man with the highest FSH, 44.3 IU/L, had bilateral cryptorchidism. Spermatozoa were obtained by needle biopsy for ICSI which resulted in a normal pregnancy.

ICSI was carried out in connection with 75 cycles of 51 couples. Spermatozoa obtained using needle biopsy were used in 49 cycles, and those from open biopsy in 29 cycles. In three cycles, spermatozoa from both needle and open biopsies were used for ICSI. The motility of the spermatozoa obtained from the biopsies was evaluated before ICSI, according to World Health Organization guidelines (WHO, 1992). The results are shown in Table I. Grade A motility was seen in only three open biopsy samples and in one needle biopsy sample.
The motility of spermatozoa derived from testicular biopsies

<table>
<thead>
<tr>
<th>Grade of motility</th>
<th>Open biopsy (no. of patients)</th>
<th>Needle biopsy (no. of patients)</th>
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<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>C</td>
<td>19</td>
<td>34</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>All</td>
<td>29</td>
<td>49</td>
</tr>
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Fertilization and pregnancy results after open and needle biopsies

<table>
<thead>
<tr>
<th></th>
<th>Open biopsy</th>
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<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>No. of ICSI cycles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injected oocytes</td>
<td>29</td>
<td>49</td>
</tr>
<tr>
<td>2PN fertilization</td>
<td>233</td>
<td>411</td>
</tr>
<tr>
<td>2+3PN fertilization</td>
<td>86</td>
<td>37</td>
</tr>
<tr>
<td>Cleaved embryos</td>
<td>92</td>
<td>39</td>
</tr>
<tr>
<td>No. of transferred embryos</td>
<td>71</td>
<td>81</td>
</tr>
<tr>
<td>No. of embryo transfers</td>
<td>48</td>
<td>75</td>
</tr>
<tr>
<td>Mean no. of embryos/transfer</td>
<td>27</td>
<td>43</td>
</tr>
<tr>
<td>HCG positive</td>
<td>1.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Clinical pregnancies</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Clinical pregnancies/embryo transfer</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>IVF pregnancies using frozen-thawed spermatozoa</td>
<td>1</td>
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PN = pronuclear; HCG = human chorionic gonadotrophin.

Embryo quality after open and needle biopsies

<table>
<thead>
<tr>
<th></th>
<th>Open biopsy (70 embryos)</th>
<th>Needle biopsy (151 embryos)</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Grade 1–2</td>
<td>56</td>
<td>79</td>
</tr>
<tr>
<td>Grade 3–4</td>
<td>14</td>
<td>21</td>
</tr>
</tbody>
</table>

*Grade 1–2, blastomeres of equal size, <20% fragmentation; grade 3–4, blastomeres of variable size, fragmentation 20–50% (grade 3) or >50% (grade 4), no degeneration.

The results regarding fertilization, embryo quality and pregnancies are presented in Table II. 233 mature oocytes were injected with spermatozoa obtained from open biopsy, and 86 (37%) of them showed normal fertilization. Normal fertilization was seen in 171 of the 411 oocytes (41%) injected with spermatozoa derived from needle biopsy. The cleavage rates (81% after open biopsy and 89% after needle biopsy) were similar in both groups. No more than two embryos at a time were transferred. An average of 1.8 embryos per transfer were transferred in 27 embryo transfers after open biopsy, and 1.7 embryos per transfer in 43 transfers after needle biopsy. Eight clinical pregnancies (29% per embryo transfer) were achieved after open biopsy, and seven (16% per embryo transfer) after needle biopsy. Seven miscarriages occurred (all during the first trimester), four in pregnancies resulting from open biopsy treatments and three from needle biopsy treatments. A total of 10 infants have been born, six singletons and two twins. The infants were healthy except one twin boy, who had Down’s syndrome. No statistically significant differences were found in the mean fertilization rates between these groups. After testing the normality (Shapiro–Wilks test), the means were compared using the two-sample t-test. Cross-tabulated data were compared using the χ² test. The numbers of cleaved embryos or clinical pregnancies did not differ significantly between these two groups. No significant differences were found in embryo quality between the two groups (Mann–Whitney U-test). The quality of the embryos is presented in Table III. Among the men with non-obstructive azoospermia, two pregnancies were achieved after needle biopsy and one after open biopsy.

In addition to fresh biopsy samples, frozen–thawed spermatozoa obtained in connection with an earlier treatment or a diagnostic procedure were used in seven treatments (three open and four needle biopsies, all obstructive azoospermia). From these, two pregnancies were achieved, one after open and one after needle biopsy. Eight additional frozen biopsy specimens (six open and two needle biopsies) were thawed but not used, because only immotile spermatozoa were found. A new needle biopsy was carried out in these cases.

Two more pregnancies have been achieved after transfer of frozen–thawed embryos to women who did not become pregnant after the first embryo transfer.

Histological diagnosis was possible from all 62 diagnostic biopsy samples taken over the same period of time. Nine of these were in connection with patients whose treatment results are included in the present study. In all, 15–20 cross-sections of seminiferous tubules per biopsied cylinder of tissue were observed (Figures 1 and 2). The costs of the total treatment procedures, which were calculated during the programme, were 32% lower in the needle biopsy group. The main additional cost of surgical biopsy arose from use of the operation theatre.

Discussion

In the present programme comprising 81 treatment cycles, the results regarding fertilization in ICSI, embryo quality and pregnancy rate were similar, using spermatozoa derived from needle biopsy and from open biopsy of the testis. No spermatozoa were obtained in six cases of non-obstructive azoospermia, a result which is comparable with those reported earlier (Tournaye et al., 1996; Friedler et al., 1997a). An earlier biopsy had not been carried out in these six men, and now we always recommend a histological needle biopsy before scheduling ICSI.

37 patients with non-obstructive azoospermia were investigated by using both fine needle (21 gauge) aspiration and open biopsy (Friedler et al., 1997a). They obtained spermatozoa by fine needle aspiration in four cases, and by open biopsy in 16 cases. Using needle biopsy, we obtained spermatozoa from all the men with obstructive azoospermia and from six of ten patients with non-obstructive azoospermia. Hence, needle biopsy appears to be at least as good as open biopsy in patients with non-obstructive azoospermia. This is supported by our histological findings, which showed 15–20 cross-sections of
Testicular biopsy gun in ICSI

Figure 1. Tubular cross-sections in a testicular needle biopsy specimen. Mature spermatids are seen in the tubules. A 5 µm paraffin section stained with haematoxylin and eosin. Original magnification ×100.

Figure 2. Cross-sections of tubules with Sertoli cells only in a testicular needle biopsy specimen. Original magnification ×100.

Seminiferous tubules per biopsy specimen. The amount of tissue is sufficient to allow reliable diagnosis and to provide enough spermatozoa for ICSI even in cases of non-obstructive azoospermia. If no spermatozoa are found on immediate examination, two or three biopsy samples can be taken from different directions through the same hole in the tunica albuginea to minimize damage to the testis. This can increase the yield of spermatozoa and minimize the risk of misdiagnosis.

Fine needle aspiration has also been used in the diagnosis of azoospermia (Forest et al., 1992; Mallidis and Baker, 1994). It gives either a cytological cell sample (Forest et al., 1992), or small fragments of tissue, with full agreement with results obtained using tissue from open biopsy in 56% and slight differences in 36% of the cases (Mallidis and Baker, 1994). Craft et al. (1997) used 19 and 21 gauge butterfly needles to obtain tissue for histological analysis. They moved the needle in a vertical direction with suction of the tissue, which they cut when removing the needle from the testis. They obtained histological samples from 20–25 tubules from 17 men. Using a biopsy gun biopsy needle, as in the present study, it is always possible to see the structure of the testicular tissue in histological examination.

It was also possible to cryopreserve spermatozoa from a needle biopsy sample, as proven by a pregnancy achieved by using spermatozoa from a frozen–thawed biopsy sample. Pregnancies have not been reported after using cryopreserved spermatozoa obtained by fine needle aspiration. Although needle biopsy is easy to repeat, it is an invasive procedure which should be avoided whenever possible. The possibility of cryopreservation is one of the advantages of needle biopsy.

Testicular damage after biopsy has been described (Schlegel and Su, 1997). There were avascular areas in the testes after biopsy, probably resulting from rupture of the arteries during the operation. They suggest that open biopsy is safer than needle aspiration because the vessels can be seen during open biopsy. However, in our experience, smaller arteries are not clearly visible and they often lie so close to the tunica that they are cut when the tunica is opened; only the largest arteries can be seen through the tunica. Knowing the anatomy of the testicular artery, it is easy to avoid the main artery under the tunica by taking a needle biopsy sample from either side adjacent to the epididymis. Because the area penetrated is very small, when compared with a knife cut, the risk of hitting an artery accidentally is lower when a needle is used. This is supported by the fact that there were no problems of bleeding or haematoma after our needle biopsies. However, colour Doppler analysis (Schlegel and Su, 1997) was not applied.

As a procedure, needle biopsy is technically simpler and
cheaper than open biopsy. It can be easily carried out in an IVF unit, which allows optimal timing as regards oocyte retrieval. Like fine needle aspiration it is much simpler than microsurgical aspiration of epididymal spermatozoa. Because it can be carried out under local anaesthesia, the patient can leave the clinic soon after the procedure.

Testicular biopsy gun biopsy gives a sufficient amount of tissue for ICSI, even in cases of non-obstructive azoospermia, for histological diagnosis and for cryopreservation. The present study was retrospective, but after needle biopsy the results regarding fertilization and pregnancy rates and the proportion of men from whom spermatozoa could be obtained were at least as good as those we achieved using open surgical biopsy. We have not compared needle biopsy directly with fine needle aspiration. According to reports published earlier (Friedler et al., 1997a), fine needle aspiration does not give as good results in non-obstructive azoospermia as open biopsy. Rosenlund et al. (Rosenlund et al., 1998) found that 21 or 19 gauge needles do not give as good histological diagnosis as open biopsies, although the method used by Craft et al. (Craft et al., 1997) allowed histological diagnosis. Testicular needle biopsy, either by biopsy gun or by butterfly needle, is the preferred methodology to open biopsy, because of its low cost, relative non-invasiveness and high success rate, with regard to the retrieval and use of testicular spermatozoa.

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References


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