Fresh versus cryopreserved testicular spermatozoa in men with nonobstructive azoospermia undergoing ICSI

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ABSTRACT

Objective: To compare the outcome of intracytoplasmic sperm injection (ICSI) with fresh and frozen-thawed testicular spermatozoa in patients with nonobstructive azoospermia.

Design: single center clinical trial

Materials and methods: Forty seven men with nonobstructive azoospermia in whom testicular sperm was found after testicular sperm extraction were enrolled (25 cases using fresh biopsy and 22 using frozen–thawed biopsy).

Main outcome measure(s): fertilization rate; mean number of embryos transferred per cycle, embryo implantation, clinical pregnancy

Result(s): There was no statistically significant difference between both groups regarding their background characteristics. There was an improved pregnancy rate with fresh versus cryopreserved testicular (P=0.053). No significant difference regarding other parameters was found.

Conclusion: In men with non obstructive azoospermia, fresh sperm extraction may yield better results than frozen biopsy.

Keywords: testicular biopsy, ICSI, nonobstructive azoospermia

Intracytoplasmic injection with testicular sperm has become a routine treatment procedure for patients with azoospermia, whether they suffer from obstructive azoospermia with normal spermatogenesis (OA) or non-obstructive azoospermia with testicular failure (NOA). High fertilization rates, pregnancy rates and implantation rates are obtained in obstructive patients (1,2). In the population of patients with NOA, however, the probability of finding sperm is only 50% in a non-selected population (3).

In contrast to OA patients, however, where viable sperm can easily be retrieved from the frozen specimens, the impaired quality of the testicular tissue of NOA patients does not allow for cryopreservation and later use for ICSI in all cases, but acceptable results of ICSI with frozen–thawed testicular sperm of NOA patients have been described (4,5). In the present study, we wished to evaluate the value of the use of frozen–thawed testicular sperm from NOA patients in an IVF program.

MATERIALS AND METHODS

Patient population

The patient cohort of this study was composed of a carefully selected group of 47 men suffering from non-obstructive azoospermia scheduled for ICSI with the use of testicular sperm. Participants were selected based upon previous testicular biopsy after semen analysis showing azoospermia.
according to WHO criteria. All couples were counseled about the procedure and signed an informed consent for their treatment. Azoospermia was confirmed on at least two diagnostic semen samples and all patients had a clinical work-up including a physical examination, hormonal assessment (FSH, LH and testosterone) and measurement of biochemical markers (Fructose) in seminal plasma.

**Intervention**

Testicular biopsies were retrieved either for diagnostic reasons followed by freezing and later ICSI cycles with frozen-thawed testicular sperm, or for immediate fresh therapeutic use (ICSI) at the day of oocyte retrieval. Participants were assigned randomly to either groups: 22 patients underwent ICSI with frozen testicular sperm while a population of 25 patients underwent ICSI cycles with fresh testicular sperm.

**Technique of testicular tissue extraction**

The surgical technique was as follows: under general anesthesia, the entire testis was delivered out through a median raphe incision. The tunica albuginea was incised and a good piece of testicular parenchyma was harvested from cranial and caudal sides on each testis with scissors. The pieces of testicular tissue thus obtained were placed in a Petri dish containing Ham's F10 medium. In the embryology laboratory, a piece from each harvested testicular tissue was minced in the Petri dish using a sterile surgical blade. The minced tissue was then checked for the presence of motile spermatozoa under an inverted microscope at 400x magnification. Enzymatic digestion was used to facilitate sperm retrieval. Whenever appropriate, additional biopsies from different regions of the testis were retrieved bilaterally. An average number of biopsies from each participants was six biopsies.

The testicular cell suspension was frozen for later use, if at least one, preferably motile, sperm was observed after diagnostic retrieval or if, after injection of the mature oocytes at the day of biopsy retrieval, sufficient remaining spermatozoa were supposed to be available for a next ICSI treatment.

**Ovarian stimulation**

Controlled ovarian stimulation was performed using long GnRH agonist down-regulation protocol. GnRH agonist (Decapeptyl 0.1 mg) was given s.c. daily starting on day 20 of the cycle. After 2-3 weeks when the down-regulation was confirmed with estradiol (E2) levels \(\geq 50\) pg/ml, hMG was started. Starting dose of hMG was 150-300 miu per day, according to the patient’s age and body weight, as well as the ovarian response in previous cycles. Monitoring was started on day 7 of hMG stimulation with daily E2 measurements and vaginal ultrasonography. Oocyte retrieval was performed 36 h after hCG administration. Embryo transfer was done on day two or three.

At the day of oocyte retrieval, testicular suspensions were thawed only when mature metaphase II oocytes were available for injection. Thawing was performed at room temperature for 5–10 min. After thawing, most spermatozoa were initially immotile but eventually resumed motility mostly in the form of tail twitching, indicating viability, after 5 h of incubation.

Luteal phase support is given to all patients who have been on analog protocols. Cyclogest (Shire Pharmaceuticals Ltd., Andover, UK) vaginal pessaries, 400mg twice a day continued for 2 weeks. B-HCG was done 2 weeks following embryo transfer and if negative Cyclogest is stopped. If, however, pregnancy test (B-HCG) was positive, Cyclogest is continued until 12 weeks gestation.

A rise in serum hCG and A clinical pregnancy was defined by the presence of a gestational sac with fetal heart beat at ultrasonography after 7 weeks of pregnancy. An ongoing clinical pregnancy was defined as a clinical pregnancy with fetal heartbeat beyond 20 weeks of pregnancy. The implantation rate is considered as the percentage of fetal sacs with heart beat on the number of embryos transferred.

**Statistical analysis**

Data are presented as mean ± SD. Different outcome measures were compared using Student's t-test or Fisher's exact test where appropriate. P values < 0.05 were considered to be significant. Statistics were done using Arcus Quickstat version 1.
Table 1. Comparison between both groups regarding different criteria and outcomes

<table>
<thead>
<tr>
<th>Item</th>
<th>Fresh</th>
<th>Frozen</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>29.7</td>
<td>29.3</td>
<td>0.48</td>
</tr>
<tr>
<td>Duration of infertility</td>
<td>5.84</td>
<td>6.3</td>
<td>0.67</td>
</tr>
<tr>
<td>Oocyte</td>
<td>12.56</td>
<td>12.47</td>
<td>0.4778</td>
</tr>
<tr>
<td>M2</td>
<td>10.15</td>
<td>10.57</td>
<td>0.38</td>
</tr>
<tr>
<td>Embryos</td>
<td>5.73</td>
<td>5.94</td>
<td>0.3828</td>
</tr>
<tr>
<td>ET</td>
<td>3.15</td>
<td>3.3</td>
<td>0.4917</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>13 / 25</td>
<td>6 / 22</td>
<td>0.053</td>
</tr>
</tbody>
</table>

RESULTS

Eleven cases were cancelled as no sperms were identified at all. Forty seven men with nonobstructive azoospermia in whom testicular sperm was found after testicular sperm extraction were enrolled (25 cases using fresh biopsy Group I and 22 using frozen –thawed biopsy Group II). There was no statistically significant difference between both groups regarding their background characteristics (Table 1). Mean age of women was 29.7 (range 19–41). Different clinical outcomes are illustrated. There were 13 conception cycles in group I (30%). In group II there were 6 conception cycles. Odds Ratio = 1.91 95% CI = 0.62 to 5.86. There was no multiple pregnancies in either group.

DISCUSSION

In the medical literature, there is a paucity of information on of using the frozen testicular suspensions for ICSI. There are also no clear-cut parameters to predict the success of sperm recovery in patients with azoospermia (6).

One major argument for using frozen testicular biopsy is the substantial risk (20%) of not finding sperm suitable for injection, despite extensive efforts which necessitates the need for a back-up fresh retrieval (7). Thus, the main advantages of cryoTESE-ICSI are to avoid repeated surgical biopsy and to ensure the availability of spermatozoa when the ovarian stimulation cycle is begun.

However, there are several draw backs for using frozen testicular biopsies. First, is the use of immotile frozen–thawed sperm for injection. In the ICSI program, motility is used as an indicator for viability. It has been shown previously that immotile fresh testicular sperm can successfully be used for ICSI (8). A reduced pregnancy rate with immotile frozen–thawed sperm was described by other investigators (9). Although in vitro culture of frozen–thawed testicular sperm may improve motility in obstructive cases, it was ineffective and unpredictable when only immotile sperm of non-obstructive cases were cultured (10,11). Despite the extremely low numbers and poor motility of testicular NOA sperm in our program, motile sperm could be found for injection in an unexpectedly high proportion of cycles.

Besides its effect on fertilization, the use of immotile sperm also affects pregnancy and implantation rates with result of high failure rate as seen in the present study where only 6 out of 22 cycles were successful (27.3%) while in the fresh biopsy group, 12 out of 25 got pregnant (48%). Although this difference between both groups was not statistically significant, but one may attribute this to the small number of our participants.

In conclusion, fresh biopsy retrieval may result in better conception rate. For each individual case, this option should be discussed beforehand between the clinician and the patient.

REFERENCES

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