Outcome of repeated testicular sperm extraction and ICSI in patients with non-obstructive azoospermia

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ABSTRACT

Objective: To evaluate the outcome of repeated TESE/ICSI, in patients with non-obstructive azoospermia, to collect sufficient information for adequate counseling of the patients.

Design: Retrospective study

Setting: The Egyptian IVF-ET center

Materials and Methods: 41 patients suffering from non-obstructive azoospermia underwent 59 repeated TESE/ICSI cycles. All of the patients had repeated TESE/ICSI because of failure to achieve pregnancy (n=30), previous abortion (n=3), or because they wanted another child (n=8). Eleven patients repeated the procedure more than once. At each procedure multiple small biopsies were taken from each testicle. The interval between the repetitions of TESE was at least six months.

Main Outcome Measures: Sperm retrieval rate in each repeated trial, and the clinical pregnancy rate per ICSI cycle

Results: During repeated TESE, the mean sperm retrieval rate was 91.5%. Patients were divided into two groups according to previous pregnancy in the first trial. In group 1, (n = 11) pregnancy occurred in the first trial. They underwent 16 TESE/ICSI repeated cycles. Of these, 9 of them became pregnant a second time (PR 56.3 %). On the other hand the 30 patients who had failed to achieve pregnancy in the first trial (Group 2), underwent 43 repeated cycles. Of these only 7 became pregnant in the following trials (16.3 %). This difference is statistically significant (X² 9.59, P< 0.05).

Conclusion: In patients with non-obstructive azoospermia, when spermatozoa were retrieved in the first TESE/ICSI cycle, the sperm recovery rate during repeated TESE trials is quite high in our study (91.5%). Previous pregnancy is a good prediction for a second pregnancy in the subsequent trials.

Keywords: Repeated TESE, Intracytoplasmic sperm injection, sperm retrieval rate,

... Intracytoplasmic sperm injection (ICSI) enables fertilization and pregnancy when only few testicular sperm are available, following surgical sperm recovery from azoospermic men. Non-obstructive azoospermia (NOA) can result from environmental events such as trauma, ionizing irradiation and exposure to cytotoxic drugs or may be primary due to genetic factor like Klinefelter's syndrome, and microdeletions of Y chromosome fragments or idiopathic. ICSI of surgically recovered sperm is the only way in which men suffering from these disorders can share the genetic parenthood of their offspring (1).

TESE may not always be successful in patients with non-obstructive azoospermia, as they only have minute foci of active spermatogenesis from which a small number of spermatozoa can be extracted. Testicular sampling evokes two important questions: the optimal number of...
biopsies that should be performed and the optimum time for repetition.

If the multiple-surgical approach is preferred, there is an increased risk of interruption of the blood supply, post-sampling fibrosis or autoimmune response (2, 3). As the detrimental effect of TESE on spermatogenesis continues for several months, it was advised not to repeat the procedure on the same testis within 6 months (2). Several surgical methods for obtaining testicular sperm needed for fertilization have evolved in different centers. The main approaches are open biopsy by testicular sperm extraction (TESE) (1, 4) and closed percutaneous testicular fine needle aspiration (TEFNA) (5, 6). TESE is performed by opening a slit in the scrotal skin and tunica albuginea, followed by excision of a small peripheral testicular tissue fragment, which is immediately processed for sperm recovery. Because sperm might be produced focally, performing biopsies at multiple sites can increase the retrieval yield of TESE (7). Open biopsies can lead to several disturbing potential side effects emanating from the apparently traumatic nature of the procedure. Bleeding, inflammation, devascularization and fibrosis, manifested both clinically and sonographically, might cause irreversible damage to the testis, producing clinical complications and limiting the possibility to repeat the procedure (2, 8). Closed TEFNA, on the other hand, is apparently less traumatic than TESE (6) but the results are poor. There are no clinical or laboratory methods that can reliably and accurately predict the presence of sperm on testicular sperm extraction. So the aim of this study was to evaluate the outcome of repeated TESE to collect sufficient information for adequate counseling of the patients with NOA.

MATERIALS AND METHODS

Study Population

This retrospective study included chart review of forty-one (41) patients suffering from non-obstructive azoospermia who underwent 59 repeated TESE/ICSI cycles during the period of October 1995 to December 1999, at the Egyptian IVF-ET Center. In all cases spermatozoa were retrieved from the testis in the first trial. TESE was repeated because cryopreservation was not done or no viable sperm were found after thawing during subsequent trials.

Examination of patients

All male partners in the study underwent physical examination and patients' history taking along with evaluation of their hormonal profile, peripheral blood karyotype and transrectal ultrasound. Diagnosis of NOA was based upon a histological report, taken during the first procedure in patients who had no diagnostic testicular biopsy prior to their first TESE.

General examination, pelvic examination, and transvaginal ultrasonography were routinely performed for the female partner. Investigations for tubal patency were only done when indicated. Routine laboratory tests included liver (SGPT and SGOT) and kidney function tests were also performed.

All of the patients had repeated TESE/ICSI because of failure to achieve pregnancy (n=30), previous abortion (n=3), or because they wanted another child (n=8). Eleven patients repeated the procedure more than once. At each procedure multiple small biopsies were taken from each testicle. The interval between the repetitions of TESE was at least six months.

Testicular biopsy

The technique of surgical testicular sperm retrieval in patients with NOA, sperm preparation and ICSI has been described in detail elsewhere (9). A conventional biopsy procedure during which multiple small biopsies were taken from each testicle was done.

Once sperm were found, the surgical procedure was terminated. If sperms were not observed, then up to three biopsies were taken, in different areas of the same testicle; followed by the contralateral testicle.

During repetitive TESE, the scrotum was opened and adhesions, if present, were dissected.
Table 1. Outcome of repeated TESE/ICSI.

<table>
<thead>
<tr>
<th>Second trial TESE</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Of cycles</td>
<td>41</td>
<td>11</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>No. of cycles with spermatozoa retrieved (%)</td>
<td>38 (92.7%)</td>
<td>10 (90.9%)</td>
<td>4 (80%)</td>
<td>2 (100%)</td>
</tr>
</tbody>
</table>

No specific technical problems were encountered in the repetitive TESE cases that could disturb the exposure of testicular tissue.

Oocyte and embryo culture

After retrieval, the oocytes were incubated in G1.2 media (Vitrolife, Motdalswaga, Goteborg) under mineral oil in tissue culture dishes (Falcon 3001) at 37°C and 5% CO2 in air. ICSI procedure was performed about 2 - 3 hours after retrieval. After about 16 - 20 hours, the oocytes were checked for PN formation and the normally fertilized ones were transferred to fresh G1.2 media that was incubated overnight and no further changes of media was done till the day of embryo transfer. The embryos were cultured in groups of 3 to 4 in 50 ml droplets of tissue culture media under mineral oil.

Embryo Transfer

On the day of ET, the best morphologically looking embryos were chosen. Selection of the embryos for transfer was based on the number of blastomeres, absence of fragmentation and the most advanced stage of development. The best 3 embryos were transferred except in patients above the age of 37 and/or with bad quality embryos, where 4 embryos could be sometimes transferred. Transfer of the embryos was done on either day 2 or day 3, according to the preference of the patient, embryologist and medical team.

Outcome measures

Two weeks after embryo transfer serum ?-hCG was measured and patients who had a positive pregnancy test were scheduled for ultrasound scan three weeks later. Clinical pregnancy was diagnosed by the presence of positive pregnancy test; in addition to ultrasonographically visible sac(s), fetal echoes and pulsations.

Statistical analysis

Statistical evaluation was performed using Student's t-test, X2-test. Differences were considered significant at P<0.05.

RESULTS

Among the 41 patients with NOA included in the study, female and male partners' age was (mean ± SD) 29.8 ± 5.9 and 33.5 ± 6.3 years respectively. Of these 41 patients, sperms were successfully retrieved in 38 cases (92.7%). Among these 38 patients, 11 underwent a third TESE trial. Spermatozoa were successfully retrieved in 10 cases (90.9%). Furthermore, 5 of these 10 patients underwent a fourth TESE trial, and sperm were found in 4 (80.0%). Two of these 4 patients repeated their TESE trial for a fifth time and was successful in both (100.0%). The overall mean sperm retrieval rate in all repeated cycles was 91.5% (Table 1).

It is our policy not to repeat the TESE procedures if no sperm are found during a previous trial. Overall in 5 patients no sperm were retrieved during a TESE procedure and therefore were counseled not to repeat the procedure (Table 1).

The patients were then divided into two groups according to previous pregnancy in the first trial. In group 1, eleven patients who became pregnant in the first trial underwent 16 TESE/ICSI repeated cycles. Of these, 9 of them became pregnant a second time (PR 56.3 %). On the other hand the 30 patients who had failed to achieve pregnancy in the first trial (Group 2), underwent 43 repeated cycles.
Of these only 7 became pregnant in the following trials (16.3%). This difference is statistically significant ($X^2 = 9.59$, $P < 0.05$).

No complications were reported during the repeated TESE except four cases who developed minor scrotal hematoma.

Overall, comparing first TESE with repetitive TESE, the difference in fertilization rate, number of oocytes injected, fertilized and cleaved did not differ significantly among the study group.

**DISCUSSION**

Information regarding the outcome of repetitive TESE procedures is scarce in the literature. To enable valuable counseling, data regarding the actual prognosis in repetitive TESE/ICSI cycles is of utmost importance, and of value even if the number of groups involved is small. Schlegel and Su recommended that TESE should be repeated at an interval of 6 months, because the chances of retrieving sperm went up to 80% compared with 25% when TESE was repeated after a shorter interval (2). Amer et al. reported their experience in repeated TESE for 27 patients with NOA (10). After performing multiple biopsies per testicle, they were able to find sperm in 24/27 (88.9%) of their patients who had sperm at their first trial. Of 19 patients who underwent the procedure after an interval of 3 months, 18 (94.7%) were positive at the repeated trial, whereas when the repeated TESE was performed after an interval of <3 months, only 6/8 (75%) were positive. Obviously the amount of testicular tissue in each patient is limited and is not regenerating after biopsies are taken and repetitive trauma to the testicles may inflict irreparable damage upon them. Ultrasonographic changes after open testicular biopsies do occur (2, 8, 10, 11); however, the clinical meaning of these findings remains to be elucidated. We found no data in the literature regarding testicular sonography after repetitive TESE. It should be recognized that absence of apparent clinical complication does not exclude possible intratesticular damage that may be demonstrated by ultrasound or that will appear at a longer interval of follow-up. Therefore, caution should be taken when counseling patients regarding repetitive TESE.

Currently, there are no clinical or laboratory methods that can predict reliably and accurately the presence of sperm on TESE. It was shown that even the presence of sperm in a preliminary testicular biopsy might fail to predict the presence of mature testicular sperm at the actual TESE/ICSI in up to 30% of cases (13). According to our results and those of others (10), consultation prior to repetitive TESE should consider that even performing TESE after the recommended interval might fail to produce mature sperm for ICSI in up to 10% of cases. Our results indicate that for patients with available sperm at their first TESE, failure to obtain sperm may occur during repetitive TESE [in our experience at a rate of 8% (3/41) up to 20% (1/5) during the second and fourth TESE respectively]. Therefore, finding of mature sperm for ICSI in the first TESE offers a good prognosis of a 90% chance of finding sperm in the following trial, but patients should be cautioned that it still may not completely assure success in further TESE trials.

In our study eleven patients who had previous pregnancy in the first trial underwent 16 TESE/ICSI repeated cycles, 9 of them became pregnant (PR 56.3%). On the other hand the 30 patients who had failed to achieve pregnancy in the first ICSI underwent 43 repeated cycles, only 7 became pregnant in the following trials (16.3%). This difference is statistically significant ($X^2 = 9.59$, $P < 0.05$).

Limiting testicular biopsy for ICSI to those with a high chance of having testicular spermatozoa has not been possible because of the poor predictive value of current clinical and laboratory methods (3, 17, 18). Tournaye et al. suggested that a preliminary testicular biopsy might be preferable in patients suffering from secretory azoospermia in order to guarantee sperm retrieval for the planned TESE/ICSI attempt (3). However, Vanderzwalmen et al. showed that in some patients with obstructive 3/115 (2.6%) and non-obstructive azoospermia 42/137 (30.6%), no spermatozoa could be found in different testicular samples at the time of the TESE/ICSI attempt, in spite of previous positive biopsies (13). Moreover, even when the testes appear clinically normal after the operation,
repeated or multiple needle or open testicular biopsies are required for diagnosis and TESE could subject the patient to potential risks of vascular injuries (2, 11).

Repetition of TESE has clinical value because pregnancies may be achieved in each repetitive trial. Previous pregnancy is a good prediction for a second pregnancy in the subsequent trials. Special difficulties or complications during or after performing the procedure were not encountered at any higher rate than after the first TESE, based on clinical examination and judgment. Due to the group's size it is difficult to draw significant conclusions regarding recommendations to perform more than four repetitive TESE procedures. New clinical tools to predict testicular mature sperm availability with proven accuracy are needed. New sperm retrieval techniques, such as micro-TESE (16) should also be tested in repeated TESE procedures in patients with NOA.

In conclusion, patients with non-obstructive azoospermia, when spermatozoa were retrieved in the first TESE/ICSI cycle, the sperm recovery rate during repeated TESE trials is quite high in our study (91.5%). Previous pregnancy is a good prediction for a second pregnancy in the subsequent trials.

REFERENCES