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·*Original Article*·

# Outcome of repeated micro-surgical testicular sperm extraction in patients with non-obstructive azoospermia

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**Abstract**

**Aim:** To evaluate the outcome of repetitive micro-surgical testicular sperm extraction (mTESE) attempts in non-obstructive azoospermia (NOA) cases, in relation to patients' initial testicular histology results. **Methods:** A total of 68 patients with NOA in whom mTESE had been performed in previous intracytoplasmic sperm injection (ICSI) attempts were reviewed. **Results:** Among the 68 patients with NOA, the first mTESE yielded mature sperm for ICSI in 44 (64%) ($Sp^+$), and failed in the remaining 24 (36%) ($Sp^-$). Following their first trial, 24 patients decided to undergo a second mTESE. Of these 24 patients, no spermatozoa were obtained in 5 patients, and $Sp^+$ but no fertilization/pregnancy were achieved in 19. In these 24 cases, mTESE was successively repeated for two ($n$ = 24), three ($n$ = 4) and four ($n$ = 1) times. The second attempt yielded mature sperm in 3/5 patients from the $Sp^-$ group and 16/19 patients from the $Sp^+$ group. At the third and fourth trials, 4/4 and 1/1 of the original $Sp^+$ patients were $Sp^+$ again, respectively. Distribution of main testicular histology included Sertoli cell-only syndrome (16%), maturation arrest (22%), hypospermatogenesis (21%) and focal spermatogenesis (41%). Overall, in repetitive mTESE, 24/29 (82%) of the attempts were finally $Sp^+$. **Conclusion:** Repeated mTESE in patients with NOA is a feasible option, yielding considerably high sperm recovery rate. In patients with NOA, mTESE may safely be repeated one or more times to increase sperm retrieval rate, as well as to increase the chance of retrieving fresh spermatozoa to enable ICSI. *(Asian J Androl 2007 Sep; 9: 668-673)*

**Keywords:** azoospermia; intracytoplasmic sperm injection; micro-surgical testicular sperm extraction; non-obstructive azoospermiarepetitive testicular sperm extraction

## 1 Introduction

Approximately 1% of all men and 10% of infertile men are affected by testicular failure as a result of obstructive or nonobstructive azoospermia (NOA) [1]. Sertoli cell-only syndrome (SCOS), maturational arrest, hypospermatogenesis and tubular sclerosis are the main histological findings of NOA. In patients with NOA, the testis is the only source of sperm cells. Mature testicular spermatozoa can be found in NOA men [2]. Testicular sperm extraction (TESE) combined with intracytoplasmic sperm injection (ICSI) offers such men the possibility of having their own genetic children. TESE with ICSI has become the standard treatment for patients with

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no source of spermatozoa except the testis [3]. However, there are no clinical or laboratory methods that can predict the presence of sperm with TESE reliably and accurately. In patients with deficient spermatogenesis as a cause of absolute or virtual azoospermia sperm, recovery rates after treatment with TESE and ICSI are reported to be 50% [4]. Also, if testicular sperm is found in patients with NOA, the pregnancy rate after one cycle of ICSI is low [5, 6], so repeated testicular biopsies combined with repetitive ICSI cycles are often needed.

There is no clear information about the recovery rates of mature spermatozoa in previous negative biopsies with the use of the microsurgical method. Therefore, in the present study we aimed to evaluate the outcome of repetitive micro-TESE (mTESE) attempts in relation to the initial testicular histology.

## 2 Materials and methods

In this retrospective study, the patient population consisted of 68 men who presented with clinical and laboratory data indicating NOA. The mean age was 36.5 (25–65) years. Azoospermia was confirmed by at least two separate seminal analyses that were carried out as described in the World Health Organization (WHO) manual [7]. Subjects underwent a full clinical evaluation including medical and reproductive histories, and physical examination of vas deferenses, epididymes and testes. Testicular size and texture were evaluated with scrotal ultrasonography. Endocrine evaluation included radioimmunoassays of serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T) (Vitros Immunodiagnostic Products; Ortho-Clinical Diagnostics, Amersham, UK). The absence of ductal obstruction was confirmed at the time of the diagnostic biopsy or at the time of mTESE by direct observation. The diagnosis of NOA was based on the finding of azoospermia in the presence of small volume testes and/or elevated serum FSH and the absence of obstruction. In all patients peripheral blood karyotype analyses were performed using standard techniques. Informed consent was obtained from all couples.

All mTESE procedures were performed by the same surgeon, in conjunction with planned *in vitro* fertilization cycles with ICSI for the female partner. Tissue samples were obtained by microdissection testicular sperm extraction technique; namely mTESE, as described previously [8]. Briefly, a 0.5–3.0 cm measuring incision was made on an avascular region of tunica albuginea, selected with the aid of optical magnification of an operating microscope (× 8). Direct examination of the testicular parenchyma was carried out at higher magnification (× 20) to identify typically dilated and more opaque seminiferous tubules. Small volumes of testicular samples, including these tubules, were extracted by traction and excised sharply. If no morphologically normal tubules were identified, the incision was extended to expose multiple areas and then any tubules that differed from the remainder of the tissue in their size or large strips of tissue when all tubules were seen to have an identical morphological appearance were removed. In cases with spermatozoon could not be extracted in one testicle, then the other one was searched by similar fashion. Surgery was stopped when spermatozoa quantitatively and qualitatively sufficient for ICSI procedure was found or when the whole testicular mass was bilaterally sampled at random. At the end of the procedure, the tunica albuginea was closed with a 5–0 vicryl suture.

After this, all testicular tissue pieces were transferred to a Petri dish filled with HEPES-buffered modified Eagle's MEM solution, and taken to the adjacent laboratory. In the laboratory, mature spermatozoa were searched for by using a mechanical extraction technique, mincing the wet preparation of testicular tissue fractions using two fine needles. When needed, erythrocyte-lysing buffer was used to increase the chance of visualizing any spermatozoa present. Microscopic examination of the suspension was carried out under an inverted microscope (Nikon, Japan) with Hoffman modulation equipment. If spermatozoa were found, then the next step was ICSI. Whenever sperm has not been found after mechanical mincing, enzymatic digestion of the testicular tissue using collagenase type IV and DNase was performed [9].

At the same time as the testicular intervention, a surgically-obtained small tissue specimen was placed in Bouin's solution and sent to histology. Biopsies were obtained unilaterally from testes in which spermatozoa were found by mechanical searching. If no spermatozoon was found, a biopsy was taken in a randomly selected area. Testicular histology was classified (as in Friedler *et al*. [10]) into hypospermatogenesis (all stages of spermatogenesis present but proportionate reduction at each level), maturation arrest (an interruption in the development of spermatogonia to mature sperm at the level of spermatogonia, spermatocytes or spermatids) and

SCOS (the absence of germ cells in the seminiferous tubules) [10]. Isolated seminiferous tubules with spermatogenetic activity observed in the field of seminiferous tubules that were otherwise maturation arrest or SCOS pattern were classified as focal spermatogenesis.

Successful mTESE was defined as the ability to find at least one spermatozoon to inject into the obtained oocyte. Patients in whom mature testicular sperm were found after the mTESE were grouped as sperm positive ($Sp^+$). Those in whom mTESE yielded no sperm available for ICSI were grouped as sperm negative ($Sp^-$). A second TESE attempt was proposed both to all sperm negative patients and to those with fertilization/pregnancy failure. All mTESE was performed by the authors of the present study.

## 3 Results

Among the 68 patients with NOA included in the study, following their first mTESE, no mature sperm was found in 24 (36%) men. The diagnoses associated with NOA in the patients are listed in Table 1. A total of seven aberrant karyotypes were diagnosed in these men, corresponding to an abnormality rate of 10%. Chromosomal abnormalities comprised one marker chromosome, four classic form of Klinefelter's syndrome with 47,XXY and two 47,XXY/46,XY mosaicism. Two patients had received chemotherapy owing to adolescent malignancies. One of them had Hodgkin's disease and the other had non-Hodgkin's lymphoma. Both patients had been treated with multiple chemotherapeutic agents containing cyclophosphamide. Comparison between the patients, regarding average age, serum FSH and T levels rendered no statistically significant difference. Successful testicular sperm retrieval rates, enabling the success of ICSI, according to the first mTESE trial are presented in Table 2. Following their first trial, 24 patients decided to undergo a second mTESE trial. Of these 24 patients, no spermatozoa had been obtained in 5 patients and $Sp^+$ but fertilization/pregnancy had not been achieved in 19.

During the 24 second mTESE attempts, in 3/5 (60%) patients from the $Sp^-$ group and 16/19 (85%) from the $Sp^+$ group, sufficient spermatozoa were found to enable ICSI (Table 3). The histology of these patients showed germ cell maturation arrest.

Further trials were performed on four patients from

Table 1. Successful sperm retrieval rates, according to associated clinical conditions. †Unilateral ($n$ = 6); bilateral ($n$ = 2); bilateral retractile ($n$ = 2); unilateral retractile ($n$ = 5).‡One marker chromosome and two Klineflter's syndrome with 47,XXY/46,XY mosaicism. TESE, testicular sperm extraction.

| Clinical Diagnosis | Number of patients (%) | Number of spermatozoa identified patients | | | |
|---|---|---|---|---|---|
| | | First TESE | Second TESE | Third TESE | Fourth TESE |
| Cryptorchidism/retractile testis | 15 (23)† | 7/15 | 5/6 | 1/1 | |
| Nontesticular neoplasia | 2 (2) | 1/2 | | | |
| Previous genital infections | 19 (28) | 16/19 | 5/7 | 1/1 | |
| Systemic lupus erythematosus | 1 (1) | 0/1 | | | |
| Exposure to X-ray | 2 (2) | 1/2 | 0/1 | | |
| Testicular trauma | 3 (4) | 2/3 | 1/1 | | |
| Nonspecific orchitis | 3 (4) | 3/3 | 2/2 | | |
| Chromosomal abnormalities | 7 (11) | 3/7‡ | 2/3 | | |
| Idiopathic | 16 (24) | 11/16 | 4/4 | 2/2 | 1/1 |
| Total | 68 | 44/68 (64) | 19/24 (79) | 4/4 (100) | 1/1 (100) |

Table 2. Successful sperm retrieval rates, according to the mTESE trials. †Overall results in 29 repetitive mTESE cases. mTSEE, micro-surgical testicular sperm extraction; TESE, testicular sperm extraction.

| mTESE trial | First TESE | Second TESE | Third TESE | Fourth TESE | Overall† |
|---|---|---|---|---|---|
| Number of patients | 68 | 24 | 4 | 1 | 29 |
| $Sp^+$ (%) | 44 (64) | 19 (79) | 4 (100) | 1 (100) | 24 (82) |

the $Sp^+$ group only. The remaining 15 $Sp^+$ cases with failed fertilization/pregnancy were excluded from the follow up because they either decided not to continue the treatment or changed hospitals. Testicular sperm was obtained by mTESE at the third trial in all four patients and at the fourth trial in one patient. Overall, in repetitive mTESE 82% of the attempts (24/29) were finally $Sp^+$ (Table 2). Correlation of the histopathological findings with sperm retrieval rates are presented in Table 4. The chances of finding testicular sperm for ICSI were significantly higher in the hypospermatogenesis group, compared with the all the other histological groups. When we divided the data for focal spermatogenesis into two groups; in approximately half of the cases (13/28; 46%) focal spermatogenesis within SCOS predominant pattern was observed; however, focal spermatogenesis within the predominant pattern of germ cell arrest was seen in the rest of the patients (54%). Regarding clinical diagnosis, in all attempts of the cases with nonspecific orchitis, testicular trauma and idiopathic etiologies, and in the majority of the attempts in cryptorchidism/retractile testis and previous genital infections, mTESE yielded successful sperm recovery for ICSI. In two of the men with aberrant karyotypes of Klinefelter syndrome with mosaicism, spermatozoa were found in repetitive mTESE attempts (Table 1). However, there was no correlation between the histopathological findings and sperm retrieval rates in successive mTESE attempts.

Intervals between the successive mTESE attempts were a minimum of 6 months. Overall, no serious complication leading to medical treatment or hospital care from any of the participants was reported. In one patient scrotal hematoma developed after his first intervention, but resolved completely in two months. After the second attempts, in two patients adhesions were encountered between the parietal layer of tunica vaginalis and tunica albuginea of testis, so that sharp dissection was necessary to reach the seminiferous tubules. During the third and fourth interventions, small hematoma formations were seen in some focal areas. However, seminiferous tubules in normal appearance were easily identified among them.

## 4 Discussion

Despite extensive damage of most of the seminiferous tubules in the biopsies of the cases with NOA, a few tubules presenting normal or nearly normal spermatogenesis can be found frequently. Currently, no clinical or laboratory methods can accurately predict the presence of sperm in testicular tissue samples. Although sperm aspiration techniques by fine needle (TESA) permits the retrieval of a large number of sperm for ICSI in patients with obstructive azoospermia, patients with NOA usually need to endure a more invasive procedure to optimize their chances of sperm retrieval. Indeed, sensitivity of fine needle aspiration cytology (FNAC) in NOA patients has been reported as 44.6% [11]. This means that if sperm are not seen by FNAC, they will be present by TESE in approximately half of the cases. However, in the majority of the patients with testicular failure spermatozoa can be extracted by TESE and used for ICSI. TESE has also been suggested as an option prior to cancer treatment in azoospermic cancer patients [15]. Several authors have proposed that mTESE be used to increase sperm retrieval rates from azoospermic patients [3]. In patients with NOA, mTESE has a significantly higher yield compared to the aspiration technique and classical open TESE [8, 10, 13].

Some couples might need a repeat TESE procedure

Table 3. Detailed correlation of the 24 previous mTESE outcomes with repetitive mTESE results. mTESE, micro-surgical testicular sperm extraction.

| mTESE trials | Previous mTESE | Repetitive mTESE (%) |
|---|---|---|
| Second | 5 ($Sp^-$) | 2 ($Sp^-$) (40) |
| | | 3 ($Sp^+$) (60) |
| | 19 ($Sp^+$) | 3 ($Sp^-$) (15) |
| | | 16 ($Sp^+$) (85) |
| Third | 4 ($Sp^+$) | 4 ($Sp^+$) (100) |
| Fourth | 1 ($Sp^+$) | 1 ($Sp^+$) (100) |

Table 4. Sperm retrieval rates during first micro-surgical testicular sperm extraction (mTESE), according to the histopathological classification. SCOS, sertoli cell-only syndrome.

| Histopathology | Number of patients | Sperm retrieval rate (*n* [%]) |
|---|---|---|
| SCOS | 11 | 3 (27) |
| Hypospermatogenesis | 14 | 14 (100) |
| Maturation arrest | 15 | 8 (53) |
| Focal spermatogenesis | 28 | 19 (67) |
| Total | 68 | 44 (64) |

on the day of oocyte retrieval to improve the chances of a second pregnancy. Information regarding the outcome of repetitive TESE procedures and the performance of ICSI is scarce in the published literature. Although cryopreservation of remaining testicular tissue is a valid option to avoid repeated surgery, cryopreservation is not always feasible in patients with NOA, because of the substantial risk of not finding sperm suitable for injection. Therefore, frozen-thawed suspensions will not be used in approximately 20% of the patients despite extensive search for sperm, and a fresh biopsy retrieval by a repeat TESE attempt needs to be carried out at the day of oocyte retrieval [21]. Donor sperm is another alternative in such cases; however, this must be agreed with the couple before the preceding treatment period. Otherwise, cancellation of the treatment is inevitable.

In the present study, a group of men with no spermatozoa who had previously unsuccessfully attempted an ICSI procedure underwent repetitive mTESE trials, with the hope of finding at least one spermatozoon. Sperm retrieval on the first TESE attempt was successful in 44 out of the 68 patients (64%). Several studies of patients with NOA reported sperm retrieval rates similar to our sperm retrieval rate (44 out of 68 patients, 64%) to be between 30% and 90% [4, 8, 10, 13, 15, 16]. However, after extensive search in the 24 remaining men (36%), no sperm was found to inject oocytes.

We found that during the second TESE procedure the sperm retrieval rate was 82% and these rates increased in the following TESE sessions if there was any sperm found during the previous TESE trials. Several published studies examine the feasibility of repeating TESE and TESA procedures in patients with primarily testicular failure. Westlander *et al*. [17] examined NOA 34 men, and of these 34, 14, 5, 3 and 1 patient(s) underwent a second, third, fourth, fifth and sixth TESA attempt, respectively. According to their data, repeating the procedure up to a sixth attempt was feasible, but histological correlation was lacking and definition of NOA was unclear. In study of Friedler *et al*. [18], 22 patients with NOA were examined and the results were correlated with corresponding histopathology. They conclude that repeating the TESE procedure up to a fourth attempt is justified. Vernaeve *et al*. [19] examined a total of 1 066 azoospermic men and suggest that repeated TESE ensures a high sperm recovery rate even in patients with NOA. Amer *et al*. [15] reported their experience in repetitive TESE for 27 NOA men. They were able to find sperm in 88.9% of their patients who had sperm at their first procedure.

Our results indicate that in 15% of the patients (3/19) with $Sp^+$ at their first TESE, failure to obtain sperm occurs during repetitive TESE. For this reason, patients should be warned that finding mature sperm for ICSI in the present TESE might not completely assure success in further TESE attempts. Similarly, according to Friedler's experience, in $Sp^+$ patients failure to obtain sperm might occur during repetitive TESE at a rate of 11% and 33% during the second and fourth TESE, respectively [18].

In the present study, five out of 24 patients with an initial negative testicular biopsy had a second mTESE and three were positive. This indicates that the outcome of repetitive TESE attempts on patients with an initial negative testicular biopsy has not been sufficiently defined and that research with a higher number of cases is necessary. Some groups do not offer a second TESE in cases of unsuccessful TESE attempts [19]. However, in three of our five patients with previous failed surgery, a second mTESE trial was attempted and sperm could be extracted. The histology of these patients had showed maturation arrest without focal spermatogenesis. During their first and second surgeries, because all tubules had an identical morphological appearance, large strips of tissues were taken randomly. However, if the number of the cases were higher, such a successful recovery rate might not be obtained. For this reason, we relate our high recovery rate of second testicular sperm extractions for patients with severe primary spermatogenic defects to the nature of the patients and, probably, to performance of the previous surgery as well as success of the tissue extraction efforts. Although the number of the patients is very small, this fact might encourage us to offer a second mTESE attempt in these patients. Supporting our results, in a study by Friedler *et al*. [18], 1/4 $Sp^-$ patients at the first TESE trial became $Sp^+$ at their second trial. However, Friedler *et al*. [18] used classical open biopsy procedure. During mTESE, sperm could not be retrieved in 33% of our cases in which mature sperm were identified at histopathological evaluation and which were described as having focal spermatogenesis. In fact, foci of active spermatogenesis on TESE can be present also in azoospermic men with testicular atrophy [11]. It was, however, shown that even the presence of mature spermatozoa in a preliminary testicular biopsy might fail to predict the pres-

ence of sperm at the successive TESE attempts at a rate of 11% and 33% during the second and fourth TESE, respectively [18]. Similar results have been reported by others [20]. According to our results, patients with histopathologically diagnosed focal spermatogenesis should be informed prior to repetitive TESE that even performing TESE might result in the failure to find mature sperm for ICSI in up to one-third of cases.

We conclude that repetitive mTESE in NOA patients is a safe and feasible option, and yields a high sperm recovery rate. Despite the absence of apparent clinical complications in our series, caution should be taken when counseling patients regarding repetitive TESE. Repetition of TESE trials obtains a considerably high sperm recovery rate to enable ICSI and has clinical value in patients with NOA, even when a first recovery procedure has been unsuccessful. However, larger series are needed to confirm the results.

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