Conventional testicular sperm extraction combined with the microdissection technique in nonobstructive azoospermic patients: a prospective comparative study

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Objective: To perform conventional and microdissection testicular sperm extraction (TESE) at the same session and compare their effectiveness.
Design: Prospective comparative study.
Setting: University hospital setting.
Patient(s): The study included 335 patients with nonobstructive azoospermia.
Intervention(s): Microdissection TESE was performed to 77 patient with atrophic testes. An additional 258 patients underwent conventional TESE using three incisions on three quadrants of the testis (upper, middle, and lower). Microdissection TESE was performed by enlarging the middle incision vertically when no spermatozoa could be detected using the conventional technique.
Main Outcome Measure(s): Sperm retrieval, fertilization, clinical pregnancy rate (PR), and live birth rate were evaluated. The relation between sperm retrieval rate and FSH level and testis volume was also investigated.
Result(s): Spermatozoa was detected in 33.7% of patients using conventional TESE. The spermatozoa detected increased to 50.8% using microdissection TESE. The increase was statistically significant. In the primary microdissection TESE group, the surgical retrieval rate was 20.8%. The overall sperm retrieval rate was 43.9%. There was a significant relation between the sperm retrieval rate and testis volume, whereas there was no relation between sperm retrieval rate and FSH levels. The overall fertilization rate, clinical PR, and live birth rate were 57.1%, 50.4%, 36.4%, respectively.
Conclusion(s): Conventional TESE combined with microdissection TESE can be used in selected patients. Sperm retrieval rate of TESE can be low in patients with atrophic testes. (Fertil Steril® 2010;94:2157–60. ©2010 by American Society for Reproductive Medicine.)

Key Words: Nonobstructive azoospermia, conventional testicular sperm extraction, microdissection testicular sperm extraction, histopathology

Nonobstructive azoospermia (NOA) refers to detecting no spermatozoa in semen analysis due to minimal or no production of fully developed spermatozoa in the testicles. Approximately 1% of all men and 10% of infertile men are affected by testicular failure as a result of NOA (1). Testicular sperm extraction (TESE) combined with intracytoplasmic sperm injection (ICSI) is a first-line treatment for infertility, including for patients with NOA (2). Such cases used to be treated with conventional TESE, including multiple biopsy samples of the testis. At present, in many clinics this treatment has been replaced by microdissection TESE. Microdissection TESE was first introduced by Schlegel in 1999 (3). This method is the ideal procedure for obtaining a high sperm retrieval rate. Direct vision with the operating microscope in microdissection TESE is of great advantage as larger, more opaque, whitish tubules, presumably containing more intratubular germ cells with active spermatogenesis, can be identified.

There have been several studies comparing conventional TESE with microdissection TESE (3–10). These studies have shown that the sperm retrieval rate (SRR) is significantly higher in microdissection TESE. In addition, the microdissection technique causes fewer preoperative and postoperative complications. However, most of the studies are retrospective, comparing various patient groups. Therefore, in this prospective study, we first performed conventional TESE and when the conventional technique failed to show spermatozoa, we turned to microdissection TESE in patients with NOA and equal testis volumes. We also evaluated histopathological features, as well as SRR, fertilization rate, clinical pregnancy rate (PR), and live birth rate, and investigated whether there was a relation between serum FSH levels and testis volume.

MATERIALS AND METHODS

The study included 335 patients with NOA who underwent TESE between September 2003 and December 2008. The presence of azoospermia was confirmed by at least two semen analyses. The patients with normal spermatogenesis, obstructive azoospermia, and hypogonadotrophic hypogonadism were excluded from the study. Also, the patients with unilateral testicular hypoplasia or atrophy (although the volume of one testis is $\geq$16 mL and the

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other is ≤5 mL) were excluded from the study. Serum FSH levels were measured in all patients. Testicular volume was measured by two clinicians (T.T. and U.G.) using a Prader orchidometer. Based on the FSH levels, the patients were divided into three groups (1–15 mIU/mL, 16–30 mIU/mL, and ≥31mIU/mL) and based on the testis volume, the patients were divided into three groups (≤5 mL, 6–15 mL, and ≥16 mL). In addition, karyotype analysis and Y chromosome gene microdeletions analysis were performed. Informed consent was obtained from all patients before the operation. Conventional and microdissection TESE procedures were performed under spinal anesthesia. Institutional Review Board (IRB) approval was obtained.

Microdissection TESE was performed on 77 patients whose testes were significantly atrophic (≤5 mL). A longitudinal incision was made to obtain a larger operation field. The remaining 258 patient underwent a conventional TESE procedure. The tunica albuginea was incised for ~5 mm at the upper pole near the head of the epididymis. If no spermatozoa were seen in the initial sample in the sperm retrieval procedure in conventional TESE, subsequent samples were taken from other locations—in the middle of the testis and at the lower pole, opposite the rete testis. When there was not a sufficient number of spermatozoa in the tubules obtained from these fields, the procedure was continued (maximum 7 biopsies) until sufficient spermatozoa were collected. The procedure was terminated when sufficient spermatozoa were retrieved. When no spermatozoa could be detected in the three poles with the conventional technique, microdissection TESE was performed by enlarging the middle incision vertically. The subtunical vessels were identified under the surgical microscope and avoided. Direct examination of the testicular parenchyma was performed at magnification ×20 to ×40 with an operating microscope. Small samples were excised from large, opaque seminiferous tubules. The procedure was terminated when a sufficient volume of spermatozoa had been retrieved for ICSI. When there were no spermatozoa in one testis, the same procedures were performed on the contralateral testis. At the same time as the testicular intervention, a surgically obtained small tissue specimen was placed in Bouin's solution and sent to the histopathology laboratory.

Sperm retrieval rate, fertilization rate, clinical PR, and live birth rate were calculated.

Statistical Analysis

Descriptive statistics were presented as mean ± SD, as well as frequencies and percentages. Analytical tests including the Student's *t*-test and the McNemar test were used to compare the two study groups, and the χ^2 test was used for categorical variables (differences were analyzed by Student's *t*-test or χ^2 test, as appropriate). The *P* value < .05 was considered to indicate a statistically significant difference.

RESULTS

The mean age of the patients was 35.2 ± 6.1 years, the mean duration of infertility was 8.0 ± 5.4 years, the mean testis size was 12.88 ± 7.2 mL, and the mean FSH concentration was 17.9 ± 12.2 mIU/mL. The SRR was 50.6% (81/160) in the patients with FSH levels of 1–15 mIU/mL, 37.7% (46/122) in the patients with FSH levels of 16–30 mIU/mL, and 37.7% (20/53) in the patients with FSH levels of ≥31 mIU/mL. There was no significant difference between the groups (*P*>.05). The SRR was 20.8% (16/77) in the patients with testis volumes of ≤5 mL, 40% (42/105) in the patients with testis volumes of 6–15 mL, and 58.2% (89/153) in the patients with testis volumes of ≥16 mL (Table 1). When testis volume increased, SRR increased significantly (*P*<.001).

The SRR was 33.7% (87/258) in the patients who underwent conventional TESE and spermatozoa were found in 44 more patients and the SRR increased to 50.8% (131/258) when the patients underwent microdissection TESE additionally. The SRR was significantly higher in the conventional and microdissection TESE group (*P*<.001). The SRR was 20.8% (16/77) in the patients who only underwent microdissection TESE. The overall SRR was 43.9% (147/335).

TABLE 1

Demographic data about patients with nonobstructive azoospermia.

Variable	Value
Age, y (mean ± SD)	35.2 ± 6.1
Spouses' age, y (mean ± SD)	30.0 ± 5.3
Infertility time, y (mean ± SD)	8.0 ± 5.4
FSH levels, mIU/mL (mean ± SD)	17.9 ± 12.2
1–15	160/335 (47.8%)
16–30	122/335 (36.4%)
≥31	53/335 (15.8%)
Testis size, mL (mean ± SD)	12.88 ± 7.2
≤5	77/335 (23%)
6–15	105/335 (31.3%)
≥16	153/335 (45.7%)

Turunc. Testicular sperm extraction in azoospermic patients. Fertil Steril 2010.

We detected spermatozoa in 147 patients. Four of them had immotile spermatozoa with severely impaired morphology and six of the patients' spouses had poor ovarian reserves. Therefore these 10 patients could not be included in the data on ICSI operations. In addition, eight patients were not included in the study because their spermatozoa were not used for ICSI. In the remaining 129 cases, the mean clinical PR was 50.4% (65/129) and the mean fertilization rate was 57.12 ± 26. Eleven patients' spouses were still pregnant at the time of the study. The live birth rate 36.4% (43/118).

The fertilization rates were 59.12 ± 26.8 and 57.85 ± 25.8, the clinical PRs were 50.6% (38/75) and 51.7% (59/114), and the live birth rates were 39.1% (27/69) and 37.1% (39/105) for conventional TESE alone and conventional TESE combined with microdissection TESE, respectively. There was no significant difference between the groups (*P*>.05). The fertilization rates, the clinical PRs, and the live birth rates for both TESE methods are presented in Table 2.

Histopathological examination showed hypospermatogenesis in 30 patients (9%), maturation arrest in 163 patients (48.6%), Sertoli cell-only syndrome in 102 patients (30.4%), and tubular sclerosis and atrophy in 40 patients (11.9%). The sperm retrieval rate was 100% (30/30) in the patients with hypospermatogenesis, 52.1% (85/163) in the patients with maturation arrest, 25.5% (26/102) in the patients with Sertoli cell-only syndrome, and 15% (6/40) in the patients with tubular sclerosis and atrophy. Histopathological features in patients with spermatozoa and those without spermatozoa after TESE are shown in Table 3.

Karyotype analysis and Y chromosome microdeletion analysis were done only in 142 patients. We could not perform genetic testing in all of the patients because of high costs and some problems with social insurance between the years of 2005 and 2007. Karyotype analysis showed nonmosaic Klinefelter syndrome in 31 patients and mosaic Klinefelter syndrome in 2 patients (23.2%). Of 31 patients, 7 (21.2%) had spermatozoa. Thirty patients with Klinefelter syndrome had testis volumes of ≤5 mL (90.9%) and three patients with Klinefelter syndrome had testis volumes of 6–15 mL (9.1%). Only six patients were diagnosed with Azfc, 1 Azfb, and 1 Azfc+d microdeletions for Y microdeletion (5.6%). Four of these patients had spermatozoa.

One patient with atrophic testis was found to have a 1-cm mass in one part of the testis during the operation and frozen sections of the mass revealed a seminoma. The patient underwent radical orchiectomy at the same session. Another patient with atrophic testis was not found

TABLE 2

Sperm retrieval, fertilization, and clinical pregnancy rates in patients with nonobstructive azoospermia.

Clinical diagnosis	Number of patients	SRR (%)	Number progress to ICSI	FR (%)	CPR (%)	Ongoing pregnancy (n)	LBR (%)
Conventional TESE	258	33.7 (87/258)	75	59.1 ± 26.8	50.6 (38/75)	6	39.1 (27/69)
Conventional TESE combined with microdissection TESE	258	50.8 (131/258)	114	57.8 ± 25.8	51.7 (59/114)	9	37.1 (39/105)
Microdissection TESE	77	20.8 (16/77)	15	51.6 ± 27.9	40 (6/15)	2	30.7 (4/13)
Total	335	43.9 (147/335)	129	57.12 ± 26.0	50.4 (65/129)	11	36.4 (43/118)

Note: SRR = sperm retrieval rate; FR = fertilization rate; CPR = clinical pregnancy rate; LBR = live birth rate; TESE = testicular sperm extraction.

Turunc. Testicular sperm extraction in azoospermic patients. Fertil Steril 2010.

to have any abnormality during microdissection TESE, but pathological examination showed Sertoli cell-only syndrome and a Leydig cell tumor. The patient had radical orchiectomy at another session.

None of the patients showed any acute or chronic complications after TESE. To determine whether the patients developed testicular failure, we measured serum T levels, but many patients did not present to our center after the operations, except in the early postoperative period. For this reason, we could not measure T levels in the late postoperative period.

DISCUSSION

Microdissection TESE is currently the best method for the definitive identification of spermatozoa, resulting in a high spermatozoa retrieval rate and minimal postoperative complications for patients with NOA (4). However, conventional TESE is still performed in the clinics where there is no operating microscope. Multiple biopsy samples from different regions of the testis and exposing spermatogenesis foci may increase the possibility of detecting spermatozoa with conventional TESE. However, it is known that it quite less likely to detect spermatozoa with conventional TESE than with microdissection TESE.

Several studies have compared the two techniques. Okada et al. (6) in their retrospective study including different patient groups, the SRR was 16.7% in the conventional TESE group and 44.6% in the microdissection group. In a study by Ramasamy et al. (9), the retrieval rate was 32% with conventional TESE and 57% with microdissection TESE. As in the present study, Okubo et al. (7) first performed conventional TESE and then microdissection TESE when conventional TESE failed to detect spermatozoa and the SRR increased from 24%–48%. However, the study by Okuba et al. had a very small sample size (n = 17) and histopathological features were not used. In a prospective comparative study of patients with NOA and bilaterally identical testicular histology who underwent conventional TESE on one testis and microdissection TESE on the other, the SRR by microdissection TESE was higher (47%) than by conventional TESE (30%). In addition, postoperative acute and chronic complications were significantly lower in the microsurgical side compared with the conventional side (8). Tsujimura et al. (5) performed salvage microdissection TESE on the patients when conventional TESE failed to show spermatozoa and reported that the SRR increased with microdissection TESE. Unlike these studies, there have been other studies showing no difference in the SRR between the two techniques (10). In fact, all of these studies, except for the one by Okubo et al., did not evaluate fertilization rate, clinical PR, and live birth rate.

Consistent with the results of the most of the studies, we found that the SRR was higher with microdissection TESE. There was no significant difference in the fertilization rates, the clinical PRs, and the live birth rates between conventional TESE alone and conventional TESE plus microdissection TESE. However, it is still remarkable that the number of clinical pregnancies increased from 38–59 and that the number of live births increased from 27–39.

Mulhall and co-workers (10) reported that the SRR was significantly higher with microdissection TESE than with conventional TESE in the patients with NOA and atrophic testis. Microdissection TESE also avoided such complications as hamatoma, fibrosis, and androgen decline, which otherwise might have been caused by conventional TESE in the patients with atrophic testes. We performed microdissection TESE on 77 patients whose testes were atrophic (testis volumes were <5 mL). Despite this effort, the SRR was only 20.8% in this group of patients.

TABLE 3

Histopathological features in patients with and without spermatozoa on TESE.

Histopathological diagnosis	Patients with spermatozoa on TESE (%)	Patients without spermatozoa on TESE (%)	All patients
Tubular sclerosis and atrophy	6/40 (15%)	34/40 (85%)	40/335 (11.9%)
Sertoli cell only	26/102 (25.5%)	76/102 (74.5%)	102/335 (30.4%)
Maturation arrest	85/163 (52.1%)	78/163 (47.9%)	163/335 (48.6%)
Hypospermatogenesis	30/30 (100%)	0/30 (0%)	30/335 (9%)
Klinefelter syndrome	7/33 (21.2%)	26/33 (78.8%)	33/142 (23.2%)

Note: TESE = testicular sperm extraction.

Turunc. Testicular sperm extraction in azoospermic patients. Fertil Steril 2010.

Although previous studies revealed a negative corrolation between increased FSH levels and the SRR, recent studies showed no significant relation between FSH levels and the SRR. Even in their study, Ramasamy and co-workers (11) reported lower SRR in the group of patients with FSH levels less than 15 IU/mL. Consistent with the literature, a significant relation between FSH levels and the SRR was not detected in our study.

It has been reported that there was no relation between the SRR and differences in testis volume among the patients with NOA who underwent TESE (12). However, we found a positive relation between the SRR and testis volume. In fact, the SRR was significantly lower (20.8%) in the patients who had testis volumes of 5mL or less. Therefore, it can be suggested that the patients with NOA whose testis volumes are lower should be informed about the low SRR with TESE. Another important issue in TESE is the amount of removed testicular tissue in the operation. The high amounts of removed testicular tissue may cause testicular insufficiency with a decrease in T levels, especially in the hypoplastic/atrophic testicles. Schlegel (3) and Amer et al. (8) reported that the amount of removed testicular tissue in microdissectional TESE was significantly lower than with the conventional method. We could not measure the amount of testicular tissue removed in patients during the TESE operation. The missing information is a possible limitation of our study.

It has been reported that the SRR is almost the same in the patients with Klinefelter syndrome as in those without Klinefelter syndrome (13). In a study by Schiff et al. (14) TESE was performed on 42 patients with Klinefelter syndrome and spermatozoa were detected in 29 patients (69%), which is even higher than the acceptable SRR in the patients with NOA. In the present study, only 21.2% of the patients with Klinefelter syndrome were found to have spermatozoa. The incidence of atrophic testis (volume $\leq$5 mL) in patients with Kleinfelter syndrome was 90.9% in our study. This rate indicates the necessity of karyotype analysis in the patients with atrophic testis. Because the patients with Klinefelter syndrome had a higher genetic risk of having abnormal children, these patients found to have spermatozoa should be offered genetic counseling (15). Microdeletions of the Y chromosome appear in 10%–15% of the patients with azoospermia (16). However, in the present study, only 5.6% of the patients with azoospermia had microdeletions of the Y chromosome. It should be kept in mind that the rate of detecting the Y chromosome in the patients with NOA may vary from region to region.

The only superiority of conventional TESE to microdissection TESE is the short operation time (4). Making a small incision in three poles of the testis with no vessels before undertaking microdissection TESE may help to access spermatozoa and shorten the operative time, which increase the effectiveness of microdissection TESE. If the tissue obtained through the small incisions did not have spermatozoa, one can start microdissection immediately. Because there may be different spermatogenesis foci in the testis, multiple biopsy samples may help to expose these foci. All things considered, performing microdissection TESE instead of conventional TESE is still the most effective treatment alternative in terms of high SRR and fewer complications.

At present, microdissection TESE is an effective sperm retrieval procedure for patients with NOA because microdissection has a greater sperm retrieval rate. However, conventional TESE combined with micodissection TESE can be performed in some patients without testicular atrophy as it may shorten the duration of the operation. One should bear in mind that the SRR is lower in patients with atrophic testis and in those with Klinefelter syndrome.

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