Comparison of Efficacy of Two Techniques for Testicular Sperm Retrieval in Nonobstructive Azoospermia: Multifocal Testicular Sperm Extraction Versus Multifocal Testicular Sperm Aspiration

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ABSTRACT: To compare the efficacy of 2 sperm-retrieval procedures, testicular sperm extraction (TESE) and testicular sperm aspiration (TESA), during the same procedure using the same subjects as their own controls. The presence of mature testicular sperm cells and motility were evaluated in 87 men with nonobstructive azoospermia (NOA) by means of multifocal TESE and multifocal TESA, which were performed during the same procedure using the same subjects as their own controls. Sperm cells were recovered by TESE in 54 cases, but by TESA in only 36 cases. There were significantly more cases (n = 20) in which sperm cells were recovered by TESE only, compared with 2 cases in whom cells were recovered by TESA only (McNemar’s test, P < .001). The mean number of locations in each testis in which sperm cells were detected was significantly higher in the TESE group. In significantly more cases (n = 27), motility was observed in TESE material only, compared with 3 cases in which motility was present in material extracted by TESA only (McNemar’s test, P < .001). Mean number of locations in each testis with motile sperm cells was significantly higher in the TESE group. The TESE procedure yielded significantly more sperm cells, as was also reflected by the difference in number of straws with cryopreserved sperm. This comparative prospective clinical study revealed that multifocal TESE is more efficient than multifocal TESA for sperm detection and recovery in men with NOA and should be the procedure of choice for sperm retrieval for them.

Key words: Male infertility, Sertoli cell only, arrest of spermatogenesis, hypospermatogenesis, motility.

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Mature sperm cells can be found in approximately 50% of the testes of men with nonobstructive azoospermia (NOA). Even when sperm cells exist, they may not be present in all the testicular samples because the testicular tissue structure may not be homogeneous, and spermatogenesis may be present only in minute foci (focal spermatogenesis) (Hauser et al, 1998). According to current standards, a man is considered sterile and cannot father his own genetic offspring if no sperm cells are detected in different locations in the testis. Under such circumstances, the use of donor sperm or adoption are the only available options remaining for the couple to have a child. It stands to reason, then, that the most reliable method should be used to reduce the chance of misdiagnosis. There is, however, no reliable noninvasive method of predicting sperm production in the testis.

Different medical groups practice a number of methods of testicular sperm retrieval, including the latest promising method of microscopic search of dilated tubules. Yet, the most common methods used are needle biopsies (testicular sperm aspiration [TESA]) and open testicular biopsy (testicular sperm extraction [TESE]). In only a few studies have both operative procedures been performed during the same procedure using the same subjects as their own controls. The outcomes of some studies were comparable (Rosenlund et al, 1998; Qublan et al, 2002; Aridogan et al, 2003), whereas others favored TESE (Friedler et al, 1997; Ezeh et al, 1998; Tournaye, 1999). Consequently, it is still unclear which is the more effective method of sperm retrieval (Van Perperstraten et al, 2001; Salihu and Alju, 2003). As was concluded in a Cochrane review: “There is insufficient evidence to recommend any specific sperm retrieval technique for azoospermic men” (Van Perperstraten et al, 2001).

The present study prospectively compared TESE with TESA in a large group of NOA men. Both procedures were performed in the same testicles during the same operation, and the results of sperm recovery were compared.
Materials and Methods

Subjects

The study cohort was comprised of 87 consecutive males with NOA who underwent testicular sperm-retrieval procedures. Men who were diagnosed as having obstructive azoospermia before the operation, based on physical examination and laboratory findings, were excluded from the study, as were those with an indefinite preoperative diagnosis and a postoperative testicular histology of normal spermatogenesis. The procedure was performed unilaterally in 7 men due to the absence of the contralateral testis (undescended or postorchiectomy). A total of 167 testes were examined in 87 patients. The men’s age ranged between 21 and 47 years. Karyotyping was performed in 83 patients, and 46XY was normal in all but 3. Two patients had XXY and another 1 had 46 Y, T(X4)(q13; p16). Y chromosome microdeletions were tested in 62 patients, and the results were positive in 6 (5 with AZF-c and 1 with AZF-B). They all presented with primary infertility of a duration ranging between 1 and 13 years and all had documentation of azoospermia in repeated semen analyses. Azoospermia was reconfirmed before the index operation by centrifugation of the ejaculate (3000 × g) for 10 minutes and a meticulous examination of the pellet under the microscope (400×). The slides were smeared with ejaculate, stained with Papanicolaou, and carefully examined under a microscope. Thirty men had normal serum follicle-stimulating hormone (FSH) concentrations (<11 mIU/mL), whereas the other 57 had elevated serum FSH levels ranging between 12 and 46 mIU/mL.

Testicular sample extraction was performed concomitantly with oocyte retrieval from the spouse, but only if the couple agreed to use donor sperm in the event that no sperm cells were found in the retrieval procedure. If the couple refused preoperative preparation of donor semen backup, the procedure of testicular sperm retrieval was performed on an elective basis, with cryopreservation for future in vitro fertilization (IVF) procedures. This policy was practiced to avoid a situation wherein there were retrieved oocytes with no available sperm.

Operative Technique

All procedures were performed by the same surgeon and with the men under general anesthesia. In all instances, the scrotum was opened via a median raphe incision and all layers were cut with meticulous hemostasis until there was full exposure of the testis. The testis and epididymis were thoroughly examined for pathological findings or for signs of obstruction. Needle aspirations (TESA) were performed in 3 different locations: in the center of the testis and in the upper and lower poles. The aim was to aspirate testicular tissue from the depth of the testis. Only after the needle was inserted into the center of the testis was a negative suction pressure applied. While maintaining negative pressure, the needle was partially withdrawn and inserted again at different angles. The sampling was performed using a needle biopsy gun that enabled a controlled and accurate sampling as well as the creation and maintenance of a substantial negative pressure during the procedure. A separate 20-mL syringe containing 0.5 mL of human tubular fluid medium (HTF, Irvine Scientific, Santa Ana, Calif) and an 18-gauge needle were used for each sample. The 3 separate samples were transferred immediately to the laboratory for sperm search and isolation.

The testicular biopsies (TESE) were performed superficially. The tunica albuginea was incised transversely for about 5 mm in 3 locations in each testis proximal to the sites of the needle sampling. The testis was then gently squeezed and the protruding tissues were excised, each weighing approximately 50 mg. Smears of each testicular biopsy were taken immediately to be used as an additional means of sperm identification and for cytological evaluation. The biopsy material was inserted into 3 separate tubes containing HTF medium and transferred to the laboratory for sperm search and isolation. The tunica albuginea was closed using 6/0 nylon monofiber, and the layers of scrotum were sutured separately.

All procedures were performed in the day-surgery clinic and lasted between 20 and 45 minutes. After recovery from anesthesia (about 2 hours), the men were discharged and advised to rest for 2 days. They all received prophylactic antibiotic treatment and were re-examined 1 week later.

Handling the Testicular Tissue and Sperm Isolation

In the laboratory, the 6 testicular tissue samples taken from each testis (3 by aspiration and 3 by dissection) were treated and examined separately. Each sample was minced using 25-gauge sterile needles. The shredded tissue was collected, centrifuged at 300 × g for 5 minutes and, after removing the supernatant, the pellet was suspended in human tubal fluid medium (Irvine Scientific) supplemented with 1% human serum albumin (Kamapharm Human Albumin; Kamada, Kibbutz Beit Kama, Israel). Using an inverted microscope and a micromanipulator, sperm cells were then identified, isolated, and aspirated into intracytoplasmic micropipettes (Humagen Fertility Diagnostics, Inc, Charlottesville, Va). After being stained with Papanicolaou (Pap), the slides of the testicular tissue smears were examined and used as another means of diagnosing the presence of sperm. The isolated sperm cells were injected into the oocytes (intracytoplasmic sperm injection [ICSI] procedure) retrieved from the spouse. Excess sperm cells and all the spermatozoa found in elective multiple TESE procedures were incubated for 3–4 hours for the acquisition of motility. They were then cryopreserved in a freezing medium (Irvine Scientific), according to the protocol published by Yogev et al (2004) in as many as 20 small aliquots containing between a few to several hundred sperm cells for future ICSI procedures.

In all instances, testicular tissue taken from a single location in each testis was also sent for histopathological evaluation by an experienced pathologist in order to exclude malignancies and to determine the histological pattern.

Evaluation of Sperm Quality

The 6 testicular samples extracted from each testis (3 by TESE and 3 by TESA) were examined separately and the results were recorded. A sample was considered positive for sperm presence even when a single mature sperm cell was found.

Because the quantity of testicular sperm cells recovered could not be measured by the same method as a routine semen analysis, we developed a scale of 1 to 3 based on the number of
cells counted per microscopic field (400x magnification): 1 = 1–10 cells; 2 = 10–100 cells; and 3 = greater than 100 cells per field.

Statistical Analysis

The outcomes between TESE and TESA were compared by paired tests: Wilcoxon nonparametric test for quantitative variables and the McNemar’s test for dichotomous variables. Pearson’s $\chi^2$ test was used to reveal the association between the different histological patterns and sperm presence, while Fisher’s exact test was used for karyotype and Y microdeletions.

Results

Genetic Characteristics

Sperm was recovered in 3 of 6 patients with Y microdeletions (all had AZF-C) and in 1 of the patients with Klinefelter syndrome. There was no significant difference in the correlations between the presence of sperm in relation to these genetic characteristics (Fisher’s exact test).

Recovery Rate of Testicular Sperm Cells

Testicular sperm cells were found in 56 of the study subjects (64.4%). The recovery methods of both TESE and TESA yielded positive results in 34/56. In the remainder, there were significantly more cases (n = 20) in which sperm cells were recovered by TESE only, compared with 2 cases for which only TESA was positive (McNemar’s test, $P < .001$; Table 1). The mean number of locations in each testis in which sperm cells were detected was also significantly higher in the TESE group (Table 2). There was a good correlation of sperm detection between retrieved samples and Pap-stained slides. Isolated morphologically abnormal sperm cells were observed only in the slides in a few of the cases. This motivated us to continue searching the testicular samples, but we failed to find any sperm cells.

The histological findings showed a mixture of patterns. Table 3 summarizes the most advanced of all the histologic patterns of each of the different subgroups of patients according to sperm-retrieval results. Sperm recovery was significantly lower in cases with a histological pattern of Sertoli cell only (SCO) or arrest of spermatogenesis compared with those with hypospermatogenesis ($P < .001$, Pearson’s $\chi^2$), but there was no significant difference between the 2 retrieval methods, TESE or TESA, for each of the different histological patterns (McNemar’s test). The histological patterns of the cases in which sperm was retrieved by TESE or TESA only were also variable. Of the 20 cases recovered by TESE only, 11 had hypospermatogenesis, 7 had SCO, and 2 showed arrest of spermatogenesis. The histologic patterns of the cases in which sperm cells were recovered by TESA only were hypospermatogenesis and SCO.

Presence of Motile Testicular Sperm Cells

Altogether, motile testicular sperm cells were detected in 48 subjects, whereas only nonmotile sperm cells were present in 8 subjects, even when both methods of sperm retrieval were performed. Motile sperm cells were detected in testicular material extracted by both TESE and TESA in 18 men. Motility was observed in TESE mate-

Table 1. Comparison of sperm recovery and presence of motility by testicular sperm extraction (TESE) and testicular sperm aspiration (TESA) retrieved from the same 167 testes in 87 men with nonobstructive azoospermia (NOA) (mean ± SD)

<table>
<thead>
<tr>
<th>Parameters Compared</th>
<th>TESE (Mean ± SD)</th>
<th>TESA (Mean ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity of sperm cells (scale of 1–3)*</td>
<td>1.1 ± 1.1</td>
<td>0.5 ± 0.7</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>No. of locations with spermatogenesis per testis</td>
<td>1.3 ± 1.3</td>
<td>0.7 ± 1.0</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>No. of locations with motile sperm cells per testis</td>
<td>0.9 ± 1.1</td>
<td>0.3 ± 0.8</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>No. of frozen straws per subject</td>
<td>4.4 ± 5.4</td>
<td>0.6 ± 1.1</td>
<td>&lt;.001†</td>
</tr>
</tbody>
</table>

* 1 indicates 1–10 sperm cells; 2, 10–100 sperm cells; 3, >100 sperm cells counted per microscopic field (400 times).
† Wilcoxon signed ranks test.
Table 4. Studies published in the literature comparing sperm recovery using both testicular sperm extraction (TESE) and testicular sperm aspiration (TESA) procedures in the same men with nonobstructive azoospermia (NOA)

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Men</th>
<th>Sperm Recovery Rate by TESE</th>
<th>Sperm Recovery Rate by TESA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(No. of Testes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosenlund et al (1998)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 gauge*</td>
<td>12 (17)</td>
<td>50% (6)</td>
<td>16.7% (2)</td>
<td>. . .</td>
</tr>
<tr>
<td>19 gauge*</td>
<td>10 (16)</td>
<td>70% (7)</td>
<td>60% (6)</td>
<td>. . .</td>
</tr>
<tr>
<td>Ezeh et al (1998)</td>
<td>35</td>
<td>63% (22)</td>
<td>14% (5)</td>
<td>&lt;.0001†</td>
</tr>
<tr>
<td>Friedler et al (1997)</td>
<td>37</td>
<td>43% (16)</td>
<td>11% (4)</td>
<td>.02‡</td>
</tr>
<tr>
<td>Tournaye (1999)</td>
<td>14</td>
<td>64.3% (9)</td>
<td>7.1% (1)</td>
<td>. . .</td>
</tr>
<tr>
<td>Qublan et al (2002)</td>
<td>27</td>
<td>33% (9)</td>
<td>30% (8)</td>
<td>NS</td>
</tr>
<tr>
<td>Aridogan et al (2003)</td>
<td>38 (76)</td>
<td>40.8% (31)</td>
<td>39.5% (30)</td>
<td>NS</td>
</tr>
<tr>
<td>Present study</td>
<td>87 (167)</td>
<td>62.1% (54)</td>
<td>24.1% (21)</td>
<td>&lt;.0001†</td>
</tr>
</tbody>
</table>

* 21- and 19-gauge needles were used in TESA.
† McNemar’s test.
‡ Fisher’s exact test.

Discussion

In the current study, we compared the efficacy of 2 widely used procedures of sperm retrieval, TESE and TESA, to contribute more clinical data to the controversial issue of which of them is more suitable for men with NOA. Our results demonstrated a significant advantage of TESE, as expressed by the sperm recovery rate (Table 1). In addition, the quantity of sperm cells, as measured directly and as reflected by the number of positive locations for sperm presence in the testis and number of frozen straws (Table 2), was significantly in favor of TESE. The same was true for the quality of sperm retrieved by both procedures, as implied by the presence of sperm motility (Tables 1 and 2). Testicular motile sperm were present in 51.7% of TESE evaluations and in 24.1% of TESA evaluations (McNemar’s test, P < .001). Sperm recovery was not different between any of the testicular histological patterns for both recovery methods, as evaluated from a single biopsy in each testis (Table 3).

The selection of study participants could substantially influence the results of a sperm-retrieval procedure. Several studies included cases with NOA as well as obstructive azoospermia (Aridogan et al, 2003). Therefore, we put special emphasis on excluding all non-NOA cases (as described in the “Material and Methods” section) and confirmed the diagnosis of NOA by testicular histology.

The most important goal of both TESE and TESA is the finding of mature sperm cells suitable for fertility treatment. In several studies, including the present one, both TESE and TESA were performed in the same testes. This enabled a valid comparison of the efficiency of the procedures in terms of detecting sperm because each man served as his own control. The TESE procedure had yielded better results in some studies (Friedler et al, 1997; Ezeh et al, 1998; Rosenlund et al, 1998; Tournaye, 1999), concurring with our results, whereas others had shown similar results for TESE and TESA (Rosenlund et al, 1998; Qublan et al, 2002; Aridogan et al, 2003). Even in such selective studies, a variety of operative techniques were used, such as different needle gauges, which may influence the results and possibly interfere with a valid comparison. Rosenlund et al (1998) performed both TESE and TESA in the same men, but they used 2 different needle sizes. As shown in Table 4, the results were similar to TESE when a thicker needle (19 gauge) was used, but they differed in favor of TESE when a 21-gauge needle was used. This may also explain the results reported by Friedler et al (1997), who used 21-gauge needles for TEFNA (TESA) and found TESE to be significantly more advantageous. This, however, cannot explain the findings of the present study where the use of 19-gauge needles still yielded inferior results for TESA compared with those of TESE. Conversely, Qublan et al (2002) used 21-gauge needles for aspiration and reported similar results for TESA and multifocal TESE. Ezeh et al (1998) performed multiple biopsies with 19-gauge needles followed by 1 open biopsy in the
same testes in 35 men and still demonstrated that TESE was significantly more efficient in sperm recovery than TESA. A less reliable method of comparison was employed in some studies, wherein TESE was performed only after failure of sperm retrieval with needle aspiration (Mercan et al, 2000; Khadra et al, 2003). These investigators also suggested that TESE was more efficient for sperm recovery.

The TESA procedure has several advantages: it is technically easier to perform and requires fewer surgical skills and training. The procedure is shorter and can be performed under local anesthesia (Belker et al, 1998; Gorgy et al, 1998). The healing process may be easier and more rapid because there are no skin scars and sutures. Nevertheless, insofar as the procedure is painful, local anesthesia or some form of sedation or even general anesthesia is always required (Gorgy et al, 1998).

On the other hand, TESE requires a fully equipped operating theater, general anesthesia, and a skillful surgeon, especially when multifocal testicular samples are extracted (Hauser et al, 1998). Moreover, the duration of the procedure is relatively short (about 20–45 minutes) and is performed on a day-clinic basis.

Complications of both procedures are relatively rare. Gorgy et al (1998) used local anesthesia for needle aspiration procedures and reported vasovagal reflex in 2 men (6%) and anxiety in 13 others (39%). It is assumed that intratesticular bleeding can be better controlled in TESE due to the full exposure of the tissue. Nevertheless, TESE is sometimes complicated by infection or hematoma (Friedler et al, 1997; Hauser et al, 1998). Both procedures may have a deleterious effect on testicular histology. A post-TESE decrease in seminiferous tubular volume within the testicular parenchyma adjacent to the biopsy site has been reported (Tash and Schlegel, 2001), and even permanent devascularization after TESE (with multifocal biopsies) was reported in isolated cases (Schlegel and Su, 1998). Moreover, the retrieval rate fell by 30% in repeated needle aspiration procedures in cases in which there had been successful sperm retrieval in the first attempt (Lewin et al, 1999). This may indicate testis damage related to the methodology of the procedure.

The TESE procedure facilitates a better histopathological evaluation of intact testicular tissue, including the peritubular space. Another advantage is the possibility of excluding testicular tumors, a rare accidental finding in azoospermic men (Yavetz et al, 1998; Schulze et al, 1999).

The comparison of the efficacy of the 2 methods, based on our results, indicate that multifocal TESE should be preferred over TESA for sperm retrieval in NOA. Other sperm-retrieval techniques that showed promising results, such as microsurgical TESE (Schlegel 1999), should also be considered for sperm retrieval in NOA patients.

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