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Prospective comparative study between microsurgical and conventional testicular sperm extraction in non-obstructive azoospermia: follow-up by serial ultrasound examinations

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The value of testicular sperm extraction (TESE) by microdissection was evaluated according to its physiological consequences compared with open, classic surgical biopsy in the same patient. A total of 100 patients with non-obstructive azoospermia and bilateral identical testicular histology underwent bilateral diagnostic TESE via the conventional method on one side and the microsurgical method on the other side. The spermatozoa recovery rate by microdissection TESE was significantly higher than by conventional TESE (47 and 30% respectively; $P < 0.05$). In order to assess the safety of this new procedure, 60 patients were followed-up ultrasonographically for 1, 3 and 6 months. Acute and chronic complications were significantly lower in the microsurgical side compared with the conventional side (15 and 58.3% respectively and 3 and 30% respectively; $P < 0.05$). Segmental devascularization was detected in seven testes operated on conventionally, and in two testes operated on microsurgically. However, permanent devascularization could not be detected in any patient after 6 months. These findings suggest that microdissection TESE is not devoid of complications, but that it is relatively safer than the conventional technique and improves sperm yield significantly in patients with non-obstructive azoospermia.

Key words: microdissection/microsurgery/non-obstructive azoospermia/sperm retrieval/testicular biopsy

Introduction

The use of testicular spermatozoa for intracytoplasmic sperm injection (ICSI) was first introduced for obstructive azoospermia in 1993 (Craft *et al*., 1993; Schoysman *et al*., 1993). It was later proved that spermatozoa can be successfully retrieved and used in ICSI in cases of non-obstructive azoospermia (NOA) (Devroey *et al*., 1995; Silber *et al.*, 1995, 1996; Mansour *et al*., 1996). Testicular sperm extraction (TESE) combined with ICSI is becoming first-line treatment in NOA. Open testicular biopsy appears to be more effective than needle biopsy for the retrieval of testicular spermatozoa in azoospermic men with defective spermatogenesis (Friedler *et al*., 1997; Ezeh *et al.*, 1998; Rosenlund *et al*., 1998). In addition, multiple TESE in such patients may be necessary as it may enhance diagnostic accuracy in absolute testicular failure and increase the number of spermatozoa retrieved (Tournaye *et al*., 1995; Hauser *et al*., 1998; Ostad *et al*., 1998). However, inflammatory changes and permanent devascularization of the testis can occur following TESE procedures with multiple biopsies (Schlegel and Su, 1997). An original study, aimed at minimizing the risk of inadvertent vascular injury to the testis, introduced the new concept of microdissection TESE which, besides maximizing the identification of the subtunical vessels, could improve sperm retrieval from men with NOA and with minimal tissue excision (Schlegel, 1999). Whether this time-consuming technique, which requires operator experience, is safer than the conventional TESE needs to be confirmed. In this study, our aim was to verify the value of such a newly introduced approach, and to evaluate the physiological consequences of the method compared with open, classic surgical biopsy in the same patient.

Materials and methods

This study was carried out on 116 azoospermic patients recruited from the outpatient andrology clinic in El Kasr EL Aini University Hospital and Adam International Clinic, both in Cairo, Egypt. Patients refusing to start an ICSI cycle without prior diagnostic TESE were offered the opportunity to undergo a bilateral diagnostic TESE with the possibility of freezing of the testicular tissues. Azoospermia was confirmed by at least two semen analyses.

A complete history and physical examinations were taken to evaluate potentially correctable causes of infertility. Hormonal evaluation included serum follicle stimulating hormone (FSH); scrotal ultrasonography was performed before surgery to evaluate testicular size and texture; the testicular blood supply was evaluated by power colour Doppler flow analysis of the testicular parenchyma. Sonographic data were obtained using an Esaote AU3 computed sonography machine with a 7.5 MHz phased array linear probe (Genoa/Florence, Italy).

Surgical approach

Testicular biopsy was carried out under general anaesthesia. Through a small vertical incision in the median scrotal raphe (2 cm), the skin, the dartos muscle and tunica vaginalis were opened to expose the tunica albuginia. The subtunical vessels were identified under the surgical microscope and avoided. A stay suture of 5/0 Prolene was placed into the tunica albuginia, after which a linear transverse (1 cm) incision was made, with care being taken to avoid subtunical vessel injury. The testicular tissues were observed under optical magnification (×24). If no morphologically normal tubules were observed, the incision was extended and blunt dissection performed between the septa of the testicular parenchyma to expose multiple areas. Copious irrigation of the field with Ringer's lactate solution was carried out

to prevent blood from obscuring the field, and a small sample was taken from the most dilated tubules, weighed and examined for the presence of spermatozoa. Another two small samples were taken from the adjacent tissues regardless of the tubular diameter; one of these was examined fresh for the presence of spermatozoa, and the other was placed in Bouin's solution for histopathological evaluation. Bipolar diathermy was applied carefully to ensure proper haemostasis. The tunica albugenia was closed using 6/0 Prolene, and the tunica vaginalis was closed with 3/0 chromic catgut. The contralateral testis was exposed via the same incision and a small incision (0.5 cm) was made in the apparently least vascular area to expose the testicular tissues. Gentle pressure was applied to the testis to extrude a sufficient amount of tissue which was excised with sharp scissors, weighed and examined for the presence of testicular spermatozoa. A second piece of tissue was taken for histopathological evaluation. The tunica albuginia was closed using 3/0 chromic catgut. The tunica vaginalis, dartos muscle and skin were closed using 3/0 chromic catgut in layers.

Sperm retrieval

The testicular tissues were taken directly into a Petri dish (Falcon, Cat. no. 3004; Becton Dickinson, Lincoln Park, NJ, USA) containing 1 ml HEPES-buffered Earle's medium. The testicular biopsy was minced and shredded using sterile glass slides and then examined immediately under an inverted microscope (Hoffman optics) using ×400 magnification for the presence and motility of testicular spermatozoa. The entire Petri dish was checked. If no spermatozoa were seen, the squeezed tissues were removed from the Petri dish and the remaining medium containing the different tissue cells was collected into 5 ml Falcon tubes. The tubes were centrifuged for 5 min at 300 *g*. The testicular pellet was resuspended in 2 ml of erythrocyte lysing buffer (Nagy *et al*., 1997) for 10 min at room temperature, after which 10 ml of HEPES-buffered Earle's medium was added to the specimen in the same tube and centrifuged for 10 min at 500 *g*. The pellet was resuspended in 50 µl of HEPES-buffered Earle's medium, after which a second search for spermatozoa was performed.

Follow-up

Ultrasonographic examinations were performed at 1, 3 and 6 months after attempts at TESE. Diffuse heterogeneity of the testicular parenchyma or focal hypoechoic lesions were considered acute. Calcifications or linear hyperechoic foci with no associated hypoechogenicity were considered chronic. Testicular blood supply was evaluated using colour Doppler flow analysis of the testicular parenchyma to detect any intratesticular devascularization. Ultrasound examinations were all performed by the same person (R.H.).

Statistical analysis

Statistical analysis was performed using an IBM computer and MS windows statistical program. Descriptive statistics were presented as mean ± SD, as well as frequencies and percentages. Analytical tests used included Student's *t*-test (two-sided) for comparing the two study groups, and χ^2 test for contingency table analysis. A *P*-value of < 0.05 was judged to be statistically significant.

Results

The study included 116 azoospermic patients. The total sperm recovery rate (SRR) was 62/116 (53%). The mean age of the patients was 33.5 ± 4.4 years, the mean duration of infertility was 4.7 ± 3.1 years, and mean FSH concentration was 15 ± 9.9 mIU/ml. Sixteen patients were excluded from the comparison as they had bilateral non-identical testicular histopathology. Among 100 patients with identical bilateral histopathology, the total SRR was 56/100 (56%). TESE was successful in all patients with normal spermatogenesis, and results in other patients are summarized in Table I. Despite removal of a significantly smaller sample of testicular tissues (4.65 ± 3.27 versus 53.57 ± 27.45 mg; $P < 0.05$), SRR was significantly higher in the side operated under optical magnification (47%) compared with the conventional side (30%; $P < 0.05$) (Table II). These results cannot be attributed to the difference in testicular histopathology on both sides, as there were no significant differences in the mean Johansen (Johansen, 1970) and late spermatid scores on both sides (Table III).

Among the 47 patients with successful TESE in the microsurgical side, 30 were found to have heterogeneous testicular tissues under optical magnification (i.e. tubules with large and small diameters). In 26 cases out of 30, spermatozoa were retrieved from the dilated tubules and no spermatozoa could be retrieved from the non-dilated tubules. In two cases, spermatozoa could be retrieved from both dilated and non-dilated tubules, and in the final two cases spermatozoa were retrieved only from the non-dilated tubules. With regard to the remaining 17 patients, testicular tissues were found to be homogeneously dilated in 15 cases and homogeneously non-

Table I. Testicular sperm recovery rates in relation to various histopathological abnormalities ($n = 100$)

Testicular histopathology	TESE
Normal histopathology	8/8 (100.0)
Hypospermatogenesis	6/7 (85.7)
Early spermatid arrest	8/10 (80.0)
Mixed pathology	22/30 (73.3)
Primary spermatocyte arrest	2/6 (33.3)
Sertoli cell-only syndrome	9/27 (33.3)
Tubular hyalinization	1/8 (12.5)
Klinefelter's syndrome (47,XXY)	0/4 (0)
Total	56/100 (56.0)

Values in parentheses are percentages.

Table II. Sperm recovery rate (SRR%) and mean weight of testicular tissue (mg) taken from the two sides operated on by microsurgery and conventional surgery ($n = 100$)

	Microsurgery	Conventional	*P*
SRR (%)	47	30	<0.05
Weight of testicular tissue removed (mg)	4.65 ± 3.27	53.57 ± 27.45	<0.05

Table III. The mean Johansen and late spermatid scores in the microsurgical and conventional sides ($n = 100$)

	Mean Johansen score	Late spermatid score
Microsurgical side	3.63 ± 2.34	1.63 ± 3.33
Conventional side	3.61 ± 2.37	1.63 ± 3.36
	$P > 0.05$	$P > 0.05$

Table IV. Intratesticular haematoma, fibrosis and segmental devascularization following microdissection and conventional TESE at 1, 3 and 6 months ($n = 60$)

Postoperative interval (months)	Microsurgical	Conventional	*P*
Hypoechoic focal lesion (haematoma)			
1	9 (15.0)	35 (58.3)	< 0.05
3	4 (6.7)	31 (51.7)	< 0.05
6	0	6 (10.0)	.....
Focal echogenic lesion (fibrosis)			
1	0	0	.....
3	0	2 (3.3)	.....
6	2 (3.3)	18 (30.0)	< 0.05
Devascularization			
1	2 (3.3)	7 (11.7)	NS
3	2 (3.3)	3 (5.0)	NS
6	0	0	.....

Values in parentheses are percentages.
NS = not significant.

dilated in two; spermatozoa could be retrieved from these patients via small microsurgical samples.

The yield of microsurgical TESE was found to be significantly higher in patients with mixed pathology, which included cases of incomplete spermatogenic arrest, incomplete Sertoli cell-only syndrome and focal tubular sclerosis [20/30 (66.6%) versus 3/30 (10%) ($P < 0.05$)]. In contrast, there was an insignificant difference between the two approaches in patients with Sertoli cell-only syndrome, primary spermatocyte arrest, early spermatid arrest, tubular hyalinization, hypospermatogenesis and Klinefelter's syndrome.

Sixty patients were followed-up for up to 6 months. Ultrasonography examinations revealed hypoechoic focal testicular lesions one month after the procedures (Table IV). After 6 months, permanent echogenic foci were found less frequently in the microsurgical side than in the conventional side ($P < 0.05$). Although colour flow Doppler imaging of the testicular parenchyma revealed segmental devascularization, no permanent testicular devascularization could be detected in any patient after 6 months (Table IV). Testicular volumes remained unchanged during the follow-up period in both sides.

Discussion

Microdissection TESE has been suggested (Schlegel, 1999) to improve sperm retrieval for men with NOA over that achieved with previously described biopsy techniques. Based on the fact that seminiferous tubules, which contain germ cells, are larger and more dilated than tubules that are devoid of these germ cells, excision of the testicular tissue can be limited with maximized yield of spermatozoa for men with NOA. In a sequential series of TESE cases for men with NOA, the ability to find spermatozoa increased from 45% (10/22) to 63% (17/27) after introduction of the microdissection technique (Schlegel, 1999). Although in our study the use of the microdissection technique increased the SRR from 30% to 47%, confirming Schlegels' results, there are some differences between the two studies. We compared the standard conventional TESE in one side and the microdissection technique on the other side in the same patient with bilateral identical histopathology. Microdissection TESE was successful in eight patients with NOA, via a relatively smaller incision (<1 cm) through which we identified and selected dilated tubules. In other patients, when no apparent healthy tubules were identified through the small incision, it was extended, but no complications were observed in such patients during the follow-up period. Because we were performing a diagnostic biopsy, we took only one sample from the conventional side and two samples from the microsurgical side; this may explain the difference in SRR between the two studies; 63% (17/27) with multiple samples (Schlegel, 1999) compared with 53% (62/116) in our study. There were insignificant differences in TESE results between the two methods in patients with spermatogenic arrest, complete tubular hyalinization, hypospermatogenesis and in Klinefelter's syndrome. Data relating to these pathological abnormalities were apparently not presented by Schlegel, who reported only that microdissection was easier when a Sertoli cell-only pattern predominates throughout the testis, and TESE was successful in only one patient with maturation arrest. We observed similar results in patients with incomplete Sertoli cell-only pattern, incomplete spermatogenic arrest and in focal tubular sclerosis ($P < 0.05$). Although Schlegel reported no apparent complications following the microdissection technique during serial ultrasound examinations after the procedures (Schlegel, 1999), we had detected testicular haematoma in 15% in the microsurgical side compared with 58.3% in the conventional side one month after TESE ($P < 0.05$). However, the incidence of permanent echogenic foci was found to be significantly lower in the microsurgical side compared with the conventional side ($P < 0.05$). It is of great importance to ensure that the microdissection technique dramatically reduced the incidence of complications and hazards of the standard open surgical TESE.

In another study (Ostad *et al*., 1998) that included 81 men with confirmed NOA, sequential biopsy attempts in a therapeutic session were carried out using optical magnifications of $\times 6$ to $\times 8$ as an approach to minimize injury during TESE. The average number of biopsy attempts was 8.9 for all patients and 6.4 for those in whom spermatozoa were isolated. These authors reported that no evidence of scrotal haematoma or haematocele formation was found postoperatively. Neither was testicular devascularization detected following their approach (Ostad *et al*., 1998). In the present study, we did not observe any scrotal haematoma or haematocele in most of the patients who had intraparenchymal haematoma during the follow-up period. Thus, the absence of clinically evident scrotal haematoma does not necessarily guarantee that testicular injury is not present.

Complications following our conventional TESE technique taking only diagnostic samples were lower than in other studies where multiple samples were taken. In an earlier study (Schlegel and Su, 1997), 64 patients were evaluated after TESE for NOA. These authors observed ultrasonographic abnormalities in the testes, suggesting resolving inflammation or haematoma at biopsy sites in 82% (14/18) of patients by one month, and parenchymal calcifications or linear hyperechoic scars in 64.2% (9/14) of patients by 6 months. Two patients had documented impaired testicular blood flow, while one patient had complete devascularization of the testis after TESE with multiple biopsies.

In our research, colour flow Doppler imaging of the testicular parenchyma revealed segmental devascularization in 7/60 testes operated on by the conventional approach, and in two testes operated on by the microdissection technique (not significant); however, no permanent testicular devascularization was detected in any testis after 6 months. This finding may be explained by the fact that patients evaluated in the former study (Schlegel and Su, 1997) were subjected to repeated TESE attempts, while our patients underwent one TESE attempt at which two testicular samples were taken. A previous study (Ron *et al*., 1998) had reported focal testicular haematoma in 20/26 (77%) one month after TESE taking up to three samples from different testicular areas. After 6 months, residual focal lesions were detected in 14/26 (54%) of the patients. These authors claimed that the residual focal lesions would be unlikely to have any adverse effect on testicular function (Ron *et al*., 1998). However, it is clear that when testicular injury is minimal—as in our study—the complications are minimal or absent. In the ICSI era, and with the need for repeated TESE attempts, there is no doubt that such therapeutic procedures would ultimately affect testicular function.

Some authors have advocated the use of biopty gun needle biopsy for extracting testicular spermatozoa from azoospermic patients. The needle can reach a wider area of testicular tissue, and so avoid the possibility that the biopsy sample is taken from a small area that is locally devoid of spermatogenesis (Rajfer and Binder, 1989; Hovatta *et al*., 1995; Tuuri *et al*., 1999). This technique appears simpler and cheaper, and can be carried out under local anaesthesia compared with our longer procedure which requires general anaesthesia. It was considered as the optimal method for the retrieval of testicular spermatozoa (Tuuri *et al*., 1999) giving sufficient amount of tissues and spermatozoa for ICSI, cryopreservation and histopathological evaluation; however, colour Doppler analysis was not performed to verify the safety of this blind procedure against the open technique. Although the microsurgical TESE could be carried out under local anaesthesia at lower cost, shorter recovery period and greater patient satisfaction (Ezeh *et al*., 1999), we preferred to use a general anaesthesia in order to avoid any possible vascular complications attributable to local spermatic cord block (Goldstein *et al*., 1983; Craft and Tsirigotis, 1995).

In conclusion, sperm retrieval is increased significantly under optical magnification, although this is not uniform in all testicular histologies. The microdissection TESE is a relatively safe procedure, but is not absolutely devoid of complications. For this reason we prefer to start with a relatively smaller tunical incision, together with meticulous identification of the dilated tubules, before extending the incision to a maximum.

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References

Craft, I. and Tsirigotis, M. (1995) Simplified recovery, preparation and cryopreservation of testicular spermatozoa. *Hum. Reprod*., **10**, 1623–1627.

Craft, I., Bennett, V. and Nicholson, N. (1993) Fertilizing ability of testicular spermatozoa. *Lancet*, **342**, 864.

Devroey, P., Liu, J., Nagy, Z.P. *et al*. (1995) Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. *Hum. Reprod*., **10**, 1457–1460.

Ezeh, U.I.O., Moore, H.D.M., Cooke, I.D. *et al*. (1998) A prospective study of multiple needle biopsies versus a single open biopsy for testicular sperm extraction in men with non-obstructive azoospermia. *Hum. Reprod*., **13**, 3075–3080.

Ezeh, U.I.O., Shepherd, S., Moore, H.D.M. *et al*. (1999) Morbidity and cost-effectiveness analysis of outpatient analgesia versus general anaesthesia for testicular sperm extraction in men with azoospermia due to defect in spermatogenesis. *Hum. Reprod*., **14**, 321–328.

Friedler, S., Raziel, A., Strassburger, D. *et al*. (1997) Testicular sperm retrieval by percutaneous needle aspiration compared with testicular sperm extraction by open biopsy in men with non-obstructive azoospermia. *Hum. Reprod*., **12**, 1488–1493.

Goldstein, M., Young, G.P.H. and Einer-Jensen, N. (1983) Testicular artery damage due to infiltration with fine gauge needle: experimental evidence suggesting that blind cord block should be abandoned. *Surg. Forum*, **24**, 653–656.

Hauser, R., Botchon, A., Amit, A. *et al*. (1998) Multiple testicular sampling in non-obstructive azoospermia – is it necessary. *Hum. Reprod*., **13**, 3081–3085.

Hovatta, O., Moilanen, J., Von Smitten, K. *et al*. (1995) Testicular needle biopsy, open biopsy, epididymal aspiration and intracytoplasmic sperm injection in obstructive azoospermia. *Hum. Reprod*., **10**, 2595–2599.

Johansen, S. (1970) Testicular biopsy score count–a method for regestiration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones,* **1**, 2–25

Mansour, R.I., Aboulghar, M.A., Serour, G.I. *et al*. (1996) Intracytoplasmic sperm injection using microsurgically retrieved epididymal and testicular sperm. *Fertil. Steril*., **12**, 566–572.

Nagy, Z.P., Devroey, P., Verheyen, G. *et al*. (1997) An improved treatment procedure for testicular biopsy specimens offers more efficient sperm recovery: case series. *Fertil. Steril*., **68**, 376–379.

Ostad, M., Liotta, D., Ye, Z. *et al*. (1998) Testicular sperm extraction (TESE) for non-obstructive azoospermia: results of multibiopsy approach with optimized tissue dispersion. *Urology*, **52**, 692–696.

Rajfer, J. and Binder, S. (1989) Use of biopty gun for transcutaneous testicular biopsy. *J. Urol*., **142**, 1021–1022.

Ron, R., Struss, S., Friedler, S. *et al*. (1998) Serial sonography and colour flow Doppler imaging following testicular and epididymal sperm extraction. *Hum. Reprod*., **13**, 3390–3393.

Rosenlund, B., Kvist, U., Ploen, L. *et al*. (1998) A comparison between open and percutaneous needle biopsies in men with azoospermia. *Hum. Reprod*., **13**, 1266–1271.

Schlegel, P.N. (1999) Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Hum. Reprod*., **14**, 131–135.

Schlegel, P.N. and Su, L.M. (1997) Physiological consequences of testicular sperm extraction. *Hum. Reprod*., **12**, 1688–1692.

Schoysman, R., Vanderzwalmen, P., Nijis, M. *et al*. (1993) Successful fertilization by testicular spermatozoa in an in-vitro fertilization program. *Hum. Reprod*., **8**, 1339–1340.

Silber, S., Van Steirteghem, A.C., Liu, J. *et al*. (1995) High fertilization and pregnancy rates after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy. *Hum. Reprod*., **10**, 148–152.

Silber, S., Van Steirteghem, A.C., Liu, J. *et al*. (1996) Normal pregnancies resulting from testicular sperm extraction and intracytoplasmic sperm injection for azoospermia due to maturation arrest. *Fertil. Steril*., **1**, 110–117.

Tournaye, H., Camus, M., Goossens, A. *et al*. (1995) Recent concepts in the management of infertility because of non-obstructive azoospermia. *Hum. Reprod*., **10**, 115–119.

Tuuri, T., Moilanen, J., Kaukoranta, S. *et al*. (1999) Testicular biopty gun needle biopsy in collecting spermatozoa for intracytoplasmic injection, cryopreservation and histology. *Hum. Reprod*., **14**, 1274–1278.

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