Comparative study between transverse and longitudinal incisions in microTESE in nonobstructive azoospermic patients

Original Article

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ABSTRACT

Background: Microdissection testicular sperm extraction (microTESE) has become the standard procedure in nonobstructive men with azoospermia. Typically, wide exposure incision is always adopted in the procedure to facilitate the identification of dilated seminiferous tubules under the microscope.

Aim: The aim was to compare transverse and longitudinal tunical incision techniques in complex nonobstructive azoospermic patients subjected to microTESE regarding the sperm-retrieval rate.

Patients and Methods: A total of 100 patients having nonobstructive azoospermia were subjected to the following: history taking, general and genital examination, semen analysis, microTESE, and testicular biopsy for histopathology. Bilateral microTESE was done through on one side by longitudinal incision technique and on the other side by transverse incision technique.

Results: Testicular sperm was successfully retrieved in 26% of patients. The sperm-retrieval rate in mixed pathology, hypospermatogenesis, spermatogenic arrest at spermatocyte level, and sertoli cell-only syndrome was 44, 60, 31, and 12.5%, respectively. This study showed no statistically significant difference in sperm retrieval between both techniques (P=0.510).

Conclusion: The microTESE technique does not affect sperm retrieval in challenging instances. This could be explained by the identical exposure of seminiferous tubules between the two techniques. However, follow-up research is required to determine whether the two procedures have fewer adverse effects on testicular parenchyma.

Key Words: Azoospermia, male infertility, spermatogenesis, TESE, testis

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INTRODUCTION

Azoospermia, defined as the lack of spermatozoa in the ejaculate after at least two evaluations of centrifuged sperm, is observed in ~1% of the population and up to 15% of infertile men. Nonobstructive azoospermia (NOA), which is found in ~60% of azoospermic men, is detectable clinically in menwith small-volume testicles, high follicle-stimulating hormone (FSH), and, of course, azoospermia[1].

Before the development of intracytoplasmic sperm injection (ICSI) and microsurgery, donor insemination was the only option for this group of individuals. However, ICSI allowed these men to use in-vitro fertilization with sperm extracted from their testicles[2].

Multiple procedures for sperm retrieval, including fine needle aspiration (FNA), percutaneous testis biopsy, open testicular biopsy or testicular sperm extraction (TESE), and microdissection testicular sperm extraction(microTESE), have been documented in the medical literature. The primary benefits of FNA and percutaneous testis biopsy procedures are their ease of use, low cost, and low invasiveness. In contrast, it has been demonstrated that the sperm-retrieval rate (SRR) was considerably lower with FNA than with traditional TESE[3]. In a conventional TESE procedure, a random incision (or incisions) in the tunica is made, and a variable tissue volume is removed to retrieve spermatozoa[4].

These repeated random tunical incisions or extensive tissue resections may result in devascularization and atrophy of the testicles. Moreover, intratesticular surgical hemorrhage and scar formation inhibit spermatogenesis and hormone synthesis[5].

Many preoperative clinical parameters, including FSH,

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age, testicular histopathology, and testicular size, have been investigated as prognostic factors for sperm retrieval in azoospermic patients; however, to our knowledge, this is the first study to compare the type of tunica albuginea incision in relation to sperm retrieval.

MicroTESE satisfies the criteria for an ideal approach for sperm extraction; it is minimally invasive, safe, and disrupts testicular function as little as possible, with a high SRR for ICSI. The testicular blood supply is observed and conserved under the supervision of an operating microscope during testicular exploration; the seminiferous tubules that are most likely to contain spermatozoa are identified and precisely targeted for extraction and sperm retrieval. In a retrospective comparison involving a small number of patients, the SRR obtained with microTESE was more significant than that obtained with traditional TESE in men with NOA, notably in the sertolicell-only (SCO) histological subtype[6]. Furthermore, microTESE has a lower complication rate than conventional testicular sperm-retrieval techniques[7].

This study aimed to evaluate the relationship between sperm-retrieval success and the type of microTESE incision in complex NOA patients.

PATIENTS AND METHODS

A total of 100 azoospermic patients from Adam Hospital for Andrology and Infertility participated in this study. Patients were chosen based on criteria indicative of NOA.

Ethical approval was obtained by the local organizing committee in a private in-vitro fertilizationcenter and from ethical committee in theFaculty of Medicine, Beni Suef University (where some of authors are affiliated).A written informed consent was obtained from all patients to participate in this work.

These criteria included at least one of the following:

(1) Repeated semen analysis showing azoospermia.
(2) Small to moderate-sized testes.
(3) High-serum FSH level.
(4) Histopathology of a previous testicular biopsy (if available).
(5) Patients had no immediate detection of sperms during extraction before tissue processing.

Exclusion criteria

Patients were excluded from the study owingto confirmation of sperm on one side (before tissue processing) or during intraoperative exploration.

Surgical procedure

TESE: microdissection technique

Under general anesthesia, the patient was positioned supine, and the scrotal and lower abdominal regions were prepared and wrappedin a sterile manner. The tunica albuginea was exposed by making a minor vertical incision in the median scrotal raphe (2cm) and opening the skin, the dartos muscle, and the tunica vaginalis. Under a surgical microscope (LEICA 500) 500 (Leica Microsystems GmbH Ernst-Leitz-Strasse 17-37 35578 Wetzlar Germany), the subtunical vessels were detected and avoided.

The transverse approach is made to one testis asfollows:a transverse incision was done extending from the hilum to the opposite side of the testis, with care being taken to avoid subtunical vessel injury[8]. Pressure is applied to expose testicular tissue. The testicular tissues were observed under optical magnification (×24). Blunt dissection was 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; samples were taken from the most dilated tubules. Sampleswere examined fresh for the presence of spermatozoa, and one of the sampleswas placed in Bouin's solution for histopathological evaluation. Bipolar diathermy was applied carefully to ensure proper hemostasis.

The longitudinal approach to the other testis is performed as follows: a linear longitudinal incision was made from the upper pole to the lower pole of the testis, taking care to avoid harm to the subtunical vascular[9]. The testicular tissues were viewed at optical magnification (×24), and the same approach was used. The tunica albuginea was closed with prolene9/0, whereas the tunica vaginalis, dartos muscle, and skin were closed in layers with vicryl 4/0.

Sperm retrieval

The testicular tissues were placed in Petri dishes (Falcon cat no 3004; Becton Dickinson, Franklin Lakes, New Jersey, USA)containing one milliliter of HEPES buffered Ham's F 10 medium (Gibco BRL, Scotland, UK). The testicular biopsy was minced using sterile glass slides and shredded with two Jeweler forceps under an Olympus stereo microscope (SZ-PT, Tokyo, Japan) with the intention of cutting and separating the individual tubules and was then immediately examined under an inverted microscope (Olympus IMT2, Tokyo, Japan) with Hoffman optics modulation and ×400 magnification power for the presence of testicular sperm. Addition of erythrocyte lysing buffer protocol is used if excess blood is obscuring the search.

Statistical analysis

Data were collected and coded to facilitate data manipulation and double entered into Microsoft Access (Product Key company Markens gate 8 4611 Kristians and Norway), and data analysis was performed using SPSS software version 18 under windows (copyright IBM corporation 2021 IBM corporation New Orchard Road Armonk,NY 10504 produced in USA May 2021).

(1) Simple descriptive analysis was in the form

of numbers and percentages for qualitative data, and arithmetic means as central tendency measurement and SDs as a measure of dispersion for quantitative parametric data.

(2) The and inferential statistic tests were performed as follows:

(a) For quantitative parametric data, paired t-test was used in comparing two dependent quantitative data.

(b) For quantitative nonparametric data, Wilcoxon tests were used in comparing two groups of dependent data.

(i) Bivariate Pearson's correlation test was used to test the association between variables.

(ii) The level *P* less than or equal *P* 0.05 was considered the cutoff value for significance.

PATIENTS AND METHODS

Our research involved 100 patients with NOA. The mean patient age was 35.7 years (range: 24–59 years), the mean infertility duration was 7.4 years (range: 1–19 years), and the average FSH level was 17.4mIU/ml (range: 2.2–48.6mIU/ml). Histopathological patterns of the examined testes included spermatogenic arrest at the spermatocyte level (29%), SCO (48%), mixed pathology (18%), and hypospermatogenesis (5%) (Tables 1 and 2). Of 100 patients who had sperm retrieval, 26 were positive. Mixed pathology and hypospermatogenesis had more excellent SRRs (44 and 60%, respectively), but spermatogenic arrest at the spermatocyte level and SCO patterns have lower SRRs (31 and 12.5%, respectively). According to our findings, the histopathological pattern significantly influenced the SRR (*P*=0.011) (Table 3).

Table 1: Testicular histopathological patterns in studied patients0

Histopathology	Frequency	%
Spermatogenic arrest at spermatocyte level	29	29.0
SCO	48	48.0
Mixed pathology	18	18.0
Hypospermatogenesis	5	5.0
Total	100	100.0

SCO, sertolicell-only.

Table 2: Sperm retrieval and testicular histopathology

	Sperm retrieval by patient		
Pathology	No	Yes	Totoal
Spermatogenic arrest at spermatocyte level			
Count	20	9	29
%	69.0	31.0	100.0
SCO			
Count	42	6	48
%	87.5	12.5	100.0
Mixed pathology			
Count	10	8	18
%	55.6	44.4	100.0
Hypospermatogenesis			
Count	2	3	5
%	40.0	60.0	100.0
Total			
Count	74	26	100
%	74.0	26.0	100.0

SCO, sertolicell-only.

Table 3: Sperm retrieval and microTESE technique

	Technique		
Pathology	Longitudinal	Transverse	Total
Spermatogenic arrest at spermtocyte level			
Sperm retrieval by testis			w
No			
Count	22	21	43
%	51.2	48.8	100.0
Yes			
Count	7	8	15
%	46.7	53.3	100.0
Total			
Count	29	29	58
%	50.0	50.0	100.0
SCO			
Sperm retrieval by testis			
No			
Count	43	43	86
%	50.0	50.0	100.0
Yes			
Count	5	5	10
%	50.0	50.0	100.0
Total			
Count	48	48	96
%	50.0	50.0	100.0
Mixed pathology			
Sperm retrieval by testis			
No			
Count	11	11	22
%	50.0	50.0	100.0
Yes			
Count	7	7	14
%	50.0	50.0	100.0
Total			
Count	18	18	36
%	50.0	50.0	100.0
Hypospermatogenesis			
Sperm retrieval by testis			
No			
Count	2	2	4
%	50.0	50.0	100.0
Yes			
Count	3	3	6
%	50.0	50.0	100.0
Total			
Count	5	5	10
%	50.0	50.0	100.0

microTESE, microdissection testicular sperm extraction; SCO, sertolicell-only.

Regarding sperm retrieval per testis, in 29 patients (58 testes) with the spermatogenic arrest at the spermatocyte level, 43 testes lacked sperm, 22 (51.2% by longitudinal incision and 21 (48.8% by transverse incision). Upon retrieval, 15 testicles contained sperm, comprising7 (46.7%) via longitudinal incision and 8 (53.3%) by transverse incision. Of 48 individuals with SCO syndrome (96 testes), sperm retrieval was successful in 10 testes, comprising five (50%) done by longitudinal incision, and five (50%) done by transverse incision. In contrast, 86 testes lacked sperm, comprising 43(50%) done by longitudinal incision, and 43(50%) done by transverse incision. A total of 18 individuals (36 testes) with mixed pathology exhibited 22 testes without sperm, comprising11(50%) by longitudinal incision and 11 (50%) by transverse incision, and 14 testes with sperm, comprising seven(50%) by longitudinal incision and seven(50%) by transverse incision. Four testes lacked sperm, comprising two(50%) by longitudinal incision and two(50%) by transverse incision, but six testes contained sperm, comprising three(50%) by longitudinal incision and three (50%) by transverse incision. There was no statistically significant difference in the SRR between longitudinal and transverse incision procedures (P=0.510) (Table 4).

Table 4: Sperm retrieval by surgical technique in different histopathological types

	Number of cases	Negative sperm	Positive sperm	Positive in both sides by both techniques	Positive in one side only
Maturation arrest at spermatocyte stage	58	43	15	14	1(transverse tunical incision)
Sertoli cell-only syndrome	96	86	10	10	
Mixed pathology	36	22	14	14	
Hypospermatogenesis	10	4	6	6	

DISCUSSION

In 1999, the microdissection technique was first described. To prevent injury to the testicular blood supply, subtunical vessels were identified using an operating microscope before biopsy incisions. Qualitative changes were discovered between the seminiferous tubules after viewing the testicular parenchyma at high magnification. This was further supported by a quantitative investigation revealing that more spermatozoa are in the bigger tubules. Initially, the testis is widened along its center in an equatorial plane. This permits broad exposure of seminiferous tubules in a healthy manner that follows intratesticular blood flow[8].

Although a longitudinal incision is also feasible within the testis, this technique has limits because the blood flow may not be clearly identified. A tiny longitudinal incision restricts testicular tissue exposure, but a longer incision may affect testicular blood flow[10].

In the isolation of sperm for intracytoplasmic sperm injection in patients with NOA, microTESE has become a recognized and successful technique (ICSI). Several criteria have been investigated and published in relation to the prediction of sperm-retrieval success rates. However, no one factor has been identified to connect with success[11].

This study aimed to examine the effect of tunica albuginea incision during microTESE on the SRR. A total of 100 patients with NOA received microTESE, with one side undergoing transverse incision and the other side undergoing longitudinal incision. In 26 of 100 patients, sperm retrieval was successful. Mixed pathology and hypospermatogenesis have a more significant percentage of sperm retrieval (44 and 60%, respectively). Still, spermatogenic arrest at the spermatocyte level and SCO patterns have a lower rate of sperm retrieval (31 and 12.5%, respectively). According to our findings, the histopathological pattern significantly affected the SRR (P=0.011).

Comparable to a 2015 study[12], our findings demonstrated that men with a diagnosis of SCO syndrome [14/35 (40%)] and maturation arrest [4/11 (36%)] had lower SRRs than those in the hypospermatogenesis group [9/12 (75.0%)]. (P<0.05).

This is comparable to the findings of Amer and colleagues; they successfully extracted sperm from 85.7% of patients with hypospermatogenesis, 80% of patients with early spermatid arrest, 73.3% of patients with mixed pathology, 33.3% of patients with primary spermatocyte arrest, 33.3% of patients with SCO, 12.5% of patients with tubular hyalinization, and 0% of Klinefelter's cases.

Our findings revealed no statistically significant difference between the two methods (P=0.510). However, just one case demonstrated sperm extraction from the testes using the transverse method. Histopathology revealed spermatogenic arrest at the spermatocyte level in this case. The quick search during surgery (before tissue processing) failed to discover any sperms, indicating that these patients

represent the most challenging situations.

CONCLUSION

The microTESE incision technique does not affect sperm retrieval in challenging instances. This could be explained by the identical exposure of seminiferous tubules between the two techniques. However, follow-up research is required to determine whether the two procedures have fewer adverse effects on testicular parenchyma.

CONFLICT OF INTEREST

There are no conflicts of interest.

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