Original Article

Comparison of sperm retrieval rate between superficial and deep dissection during microscopic testicular sperm extraction

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Abstract

Objective: The purpose of the study was to compare the outcome of microscopic testicular sperm extraction (micro-TESE) between superficial and deep dissection on the same testicle in terms of sperm retrieval rate (SRR).

Patients and Methods: In a retrospective study from June 2019 to October 2021, 44 patients with nonobstructive azoospermia who underwent micro-TESE with positive results (mature sperm identified) were included. Eight patients were excluded from the study due to deficient documentation on superficial and deep dissection. A total of 36 patients were included; 60 testicles were examined for superficial and deep biopsies. Testicular histopathology was performed in all patients, and a hormonal evaluation was obtained before the micro-TESE attempt.

Results: Thirty-six patients and 60 testicles were included in the study. Of them, 47 (78.3%) testicles had positive results. Superficial TESE was positive in 38 (63.3%) testicles, and deep TESE was successful in 45 (75.0%) testicles. An improvement of 13.9% in the SRR was observed, following deep dissection. However, there was no statistically significant difference ($P = 0.166$). Rates of positive sperm retrieval (from any side) did not differ significantly based on patients' age, microdissection testicular sperm extraction sides, and hormonal concentrations; these differences were not apparent after superficial or deep TESE.

Conclusion: The presented findings suggest that although successful SRRs of deep TESE were higher than that of its superficial counterpart, there was no significant statistical difference. A larger body of evidence is needed to provide a higher grade of recommendation.

Keywords: Azoospermia, infertility, microdissection testicular sperm extraction, sperm retrieval

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INTRODUCTION

Primary testicular failure accounts for 10% of all infertile men with azoospermia, and in which 70% of the reported cases are nonobstructive azoospermia (NOA).[1,2] Evidence has shown that conventional testicular sperm extraction (cTESE) is superior to single testicular biopsy in terms of sperm retrieval rate (SRR). However, for the assessment of male infertility, a single testicular biopsy was considered a standard diagnostic tool.[3]

Testicular parenchyma heterogeneity has been observed in most of the men with NOA, and therefore, Schlegel *et al.* have described a new technique in 1997 using the surgical microscope with a magnification power of 25 folds that can isolate foci in spermatogenesis.[4]

In addition, using surgical microscopy, evidence has shown that microdissection is more reliable in terms of SRR with up to 66% compared to cTESE.[3,5] Furthermore, it carries fewer complication rates, resulting in less tissue removal with better tissue quality, and due to its magnification and visual enhancement, it helps in the identification of small blood vessels within the testicle and thus minimizing the risk for vascular injury.[6]

To preserve testicular function and avoid complications and also to reduce the operative time, many surgeons try to avoid deep extensive tissue dissection. It is unknown if the biopsy location inside the testis during microscopic testicular sperm extraction (micro-TESE) can predict the presence of sperm and if there is any difference in the sperm retrieval rate (SRR) between superficial and deep biopsy.

From this point, we conducted this retrospective study to investigate if there is a difference in the outcome of micro-TESE between superficial and deep biopsies on the same testicle in terms of SRR.

PATIENTS AND METHODS

From June 2019 to October 2021, 44 patients with NOA who underwent micro-TESE with positive results (mature sperm identified) were included. Of them, eight patients were excluded from the study due to deficient documentation. A total of 36 patients (60 testicles) were examined for superficial and deep biopsies.

Testicular histopathology was performed in all patients by taking random testicular parenchyma samples during the dissection. Moreover, preoperatively, a complete hormonal panel was obtained and referenced.

The micro-TESE procedure was performed as described by Schlegel and Li, 1998.[7]

Directly after tunical incision and before bivalving the testis, superficial dissection of testicular parenchyma was performed under an operative microscope (at magnification ×10–×24) aiming to locate and collect the larger seminiferous tubules [Figure 1]. Then, the specimen was sent separately to the *in vitro* fertilization (IVF) laboratory labeled as "superficial testicular biopsy."

Then, the testicular parenchyma was widely exposed in its equatorial plane bluntly using the operator's fingers with attention to preserving the subtunical vessels, and tubules were retrieved deeply from different sites (upper and lower poles) of the two testicular sections trying to cover all deep testicular compartments down to rete testis (posterior tunica) [Figure 2]. Then, the specimen was sent separately to the IVF laboratory labeled as "deep testicular biopsy."

Data were analyzed using RStudio (R version 4.1.1, Posit, Boston, United States). Categorical data were presented using frequencies and percentages, whereas numerical data were expressed using medians and interquartile ranges (IQRs). Factors associated with sperm retrieval after superficial and deep TESE (on the patient level) were assessed using a Wilcoxon rank-sum test for numerical variables or a Fisher's exact test for categorical variables. The statistical differences in the positive retrieval rates in superficial and deep TESE were assessed using a Fisher's exact test or a Pearson's Chi-squared test whenever appropriate. Statistical significance was deemed at $P < 0.05$.

RESULTS

Characteristics of patients and their association with sperm retrieval

Data of 36 patients were analyzed in the current study. The median (IQR) age of patients was 39.5 (34.8–41.2) years. Two patients (5.6%) had Klinefelter syndrome. The median concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were 10.6 (7.8–16.2), 17.4 (7.9–25.7), and 13.8 (9.7–16.5), respectively. Almost two-thirds of patients underwent bilateral TESE procedures (66.7%). Successful sperm retrieval was attained for 83.3% of patients who underwent superficial microdissection testicular sperm extraction (M-TESE) and 97.2% who underwent deep M-TESE [Table 1]. The rates of positive sperm retrieval (from any side) did not differ significantly based on patients' age, TESE sides, and hormonal concentrations;

these differences were not apparent after superficial or deep TESE [Table 2].

Analysis of sperm retrieval based on individual testicles

A total of 60 testicles were included in the current analysis. More than half of the M-TESE procedures were performed on the left testicles (51.7%), whereas 48.3% of procedures were performed on the right side (48.3%). Superficial TESE was positive in 38 (63.3%) testicles, and deep TESE was successful in 45 (75.0%) testicles. The most common histopathological findings in the testicles under study were the Sertoli cell-only patterns (46.7%), maturation arrest at primary spermatocytes (31.7%), and tubular hyalinization [31.7%, Figure 3].

The histopathology distribution among patients with positive sperm retrieval in both superficial and deep dissection was 11 (30.5%) patients with SCO, 10 (27%) patients with MA, and 15 (41.6%) patients with hypospermatogenesis. Whereas, patients with positive deep dissection only showed 3 (22.2%) patients with hypospermatogenesis, 3 (22.2%) patients with MA, and 5 (55.5%) patients with SCO. In patients with positive superficial dissection only, 1 (50%) patient had hypospermatogenesis, and 1 (50%) patient with MA. There were no significant differences in successful sperm retrieval by superficial and deep M-TESE in terms of the histopathological findings [Table 3].

Table 1: Demographic and clinical characteristics

Parameter	Category	*n*=36
Age	Num	39.5 (34.8–41.2)
FSH	Num	17.4 (7.9–25.7)
Testosterone	Num	13.8 (9.7–16.5)
LH	Num	10.6 (7.8–16.2)
E2	Num	101.0 (73.2–123.0)
KF, *n* (%)	No	19 (52.8)
	Yes	2 (5.6)
	NA	15 (41.7)
TESE side, *n* (%)	Left	6 (16.7)
	Right	6 (16.7)
	Bilateral	24 (66.7)
Successful retrieval superficial, *n* (%)	Yes	30 (83.3)
Successful retrieval deep, *n* (%)	Yes	35 (97.2)

FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TESE: Testicular sperm extraction, KF: Klinefelter Syndrome, Num: Number

The association between the microdissection testicular sperm extraction sperm method and the retrieval outcome

Superficial M-TESE was positive in 38 (63.3%) testicles, and deep M-TESE was successful in 45 (75.0%) testicles. In 36/47 (76.5%) testicles, both superficial and deep dissection were positive for sperms. On the other hand, 9/47 (19.1%) testicles had sperm identified in the deep testicular dissection while superficial dissection was negative for sperm. 2/47 (4.2%) testicles had sperm identified in the superficial testicular dissection while deep dissection was negative for sperm. There was no significant difference in the rate of sperm retrieval between superficial and deep TESE [$P = 0.166$, Table 4].

DISCUSSION

In this study, positive sperm retrieval micro-TESE was identified in 36 patients (47 testicles) with NOA, which were examined for superficial and deep testicular dissection. Successful sperm retrieval was attained for 83.3% of patients who underwent superficial M-TESE and 97.2% who underwent deep M-TESE. An improvement of 13.9% in the SRR was observed, following deep dissection. However, there was no statistically significant difference ($P = 0.166$). To identify which subgroup of patients would benefit most from extensive deep dissection, serum FSH, testosterone, and histopathology distribution were evaluated. The rates of positive sperm retrieval

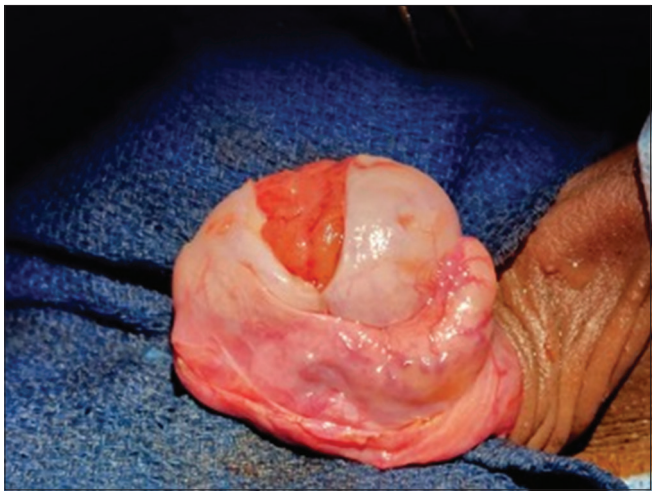

Figure 1: Superficial dissection

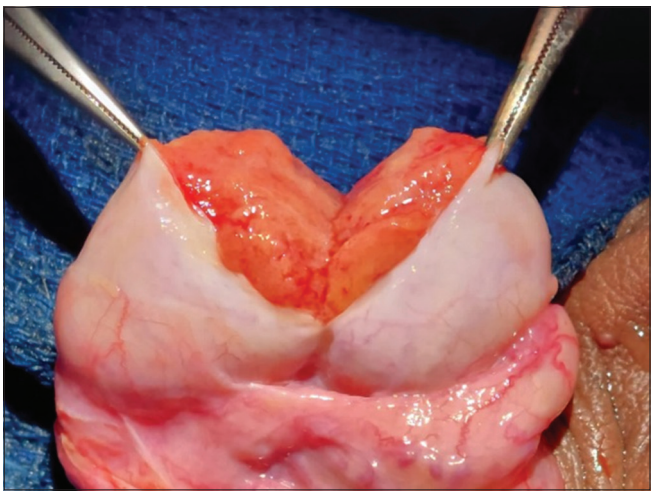

Figure 2: Superficial dissection

Table 2: Factors associated with positive sperm retrieval after superficial and deep testicular sperm extraction among patients under study (*n*=36)

Parameter	Positive retrieval on superficial TESE			Positive retrieval on deep TESE		
	No (*n*=6)	Yes (*n*=30)	*P*	No (*n*=1)	Yes (*n*=35)	*P*
Age	36.0 (30.5–43.8)	39.5 (35.2–41.0)	0.551	41.0 (41.0–41.0)	39.0 (34.5–41.5)	0.530
FSH	18.4 (8.6–45.3)	17.4 (8.7–24.9)	0.538	3.7 (3.7–3.7)	18.0 (9.3–26.4)	0.149
Testosterone	10.2 (7.2–12.6)	14.3 (10.3–17.3)	0.071	30.5 (30.5–30.5)	13.7 (9.7–16.1)	0.102
LH	12.9 (8.0–23.8)	10.6 (7.6–15.0)	0.458	1.0 (1.0–1.0)	10.6 (8.0–16.2)	0.123
E2	71.4 (70.7–94.0)	107.0 (77.4–130.0)	0.150	156.0 (156.0–156.0)	97.5 (72.3–118.2)	0.216
KF, *n* (%)	1 (16.7)	1 (3.3)	0.310	0	2 (5.7)	>0.999
TESE side, *n* (%)						
Left	0	6 (20.0)	0.804	0	6 (17.1)	>0.999
Right	1 (16.7)	5 (16.7)		0	6 (17.1)	
Bilateral	5 (83.3)	19 (63.3)		1 (100.0)	23 (65.7)	

FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TESE: Testicular sperm extraction, KF: Klinefelter Syndrome

Table 3: The association between histopathological findings and sperm retrieval by testicular sperm extraction procedures in the testicles under study (*n*=60)

Histopathological findings	Positive retrieval on superficial TESE			Positive retrieval on deep TESE		
	No (*n*=22), *n* (%)	Yes (*n*=38), *n* (%)	*P*	No (*n*=15), *n* (%)	Yes (*n*=45), *n* (%)	*P*
No evidence of spermatogenesis	0	5 (13.2)	0.148	0	5 (11.1)	0.318
Hypospermatogenesis	5 (22.7)	13 (34.2)	0.350	5 (33.3)	13 (28.9)	0.754
Maturation arrest at primary spermatocytes	7 (31.8)	12 (31.6)	0.985	6 (40.0)	13 (28.9)	0.525
Sertoli cell-only pattern	11 (50.0)	17 (44.7)	0.694	8 (53.3)	20 (44.4)	0.550
Prominence of Leydig cells	3 (13.6)	4 (10.5)	0.700	1 (6.7)	6 (13.3)	0.668
Tubular hyalinization	10 (45.5)	9 (23.7)	0.081	5 (33.3)	14 (31.1)	>0.999
Tubular atrophy	1 (4.5)	4 (10.5)	0.643	1 (6.7)	4 (8.9)	>0.999

TESE: Testicular sperm extraction

Table 4: The association between the testicular sperm extraction sperm method and the retrieval outcome

Parameter	Category	TESE procedure		*P*
		Deep, *n* (%)	Superficial, *n* (%)	
Outcome	Negative	15 (25.0)	22 (36.7)	0.166
	Positive	45 (75.0)	38 (63.3)	

TESE: Testicular sperm extraction

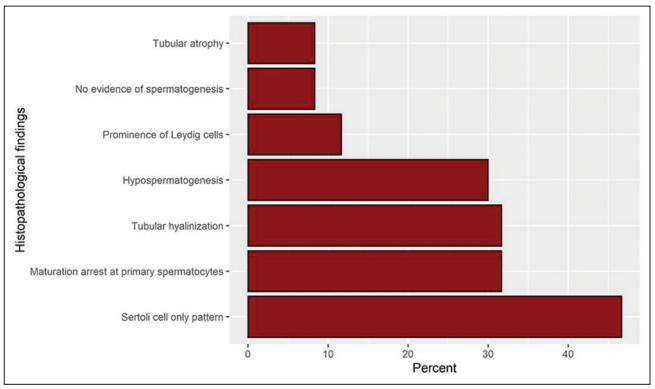

Figure 3: The percentages of histopathological findings indicated in the testicles under study (*n* = 60)

(from any side) did not differ significantly based on patients' age, M-TESE sides, and hormonal concentrations; these differences were not apparent after superficial or deep M-TESE.

Our results showed that the chance to have positive sperm in deep dissection, following a positive superficial dissection, is 76.5%; however, there was a significant proportion of positive deep dissection specimens that had a previously negative superficial dissection (19.1%). In addition, there is a small chance to find sperms in the superficial dissection with a negative deep dissection (4.2%). Hence, superficial testicular dissection alone during micro-TESE could fail to reveal sperm in up to a fifth of the men with NOA.

To identify which subgroup of patients would benefit most from extensive deep dissection, serum FSH, testosterone, and histopathology distribution were evaluated. Our study demonstrates that although not significant, men with lower serum FSH, higher Serum testosterone, and hypospermatogenesis on preoperative diagnostic biopsy had a higher chance of finding sperm on initial superficial dissection. While in men with higher serum FSH, lower testosterone, and SCO on preoperative diagnostic biopsy, the chance of finding sperm during micro-TESE will improve by extensive deep dissection.

In our view, it is reasonable to believe that a possible reason for the failure to identify sperm in micro-TESE could be that tubule dissection during the procedure remained superficial. Following our experience, we observed many positive redo micro-TESE done in our center after failure to identify sperm in the first micro-TESE done outside, which could be due to a lack of deep dissection during the first micro-TESE. A study strategy similar to ours has been

applied by Ramasamy *et al*., reporting their technique of micro-TESE starting with a superficial extraction of tubules, followed by a deeper and more extensive search below the superficial section.[8] This second step improved the SRR by 18.4%.[8] Our results replicate this finding with a 13.9% SRR improvement after the second deep dissection step.

Micro-TESE with superficial dissection is less invasive than deep dissection, as superficial dissection avoids detachment of the subtunical vessels and allows a sparing of the intratesticular vessels with minimal excision of testicular parenchyma. Theoretically, it is associated with fewer complications such as intratesticular hematoma, testicular scarring, atrophy, and hypogonadism compared with deep dissection. To the best of our knowledge, there is no study conducted to compare the complication outcome of deep testicular dissection with superficial dissection Micro-TESE on a large scale. Many comparative studies were conducted to compare the complication rates between conventional and micro-TESE, which have shown that micro-TESE carries fewer changes in the microdissection group compared to the conventional group.[9-11]

Donoso *et al*. found that 80% of patients who underwent TESE had structural changes or intratesticular hematoma on the postoperative ultrasound compared to Amer *et al*., who found a 30% rate of structural changes and a 3.3% fibrosis rate in micro-TESE patients.[9,12] In addition, many studies have been conducted to compare cTESE and micro-TESE in decreased serum testosterone levels postoperatively. In a large survey, both cTESE and micro-TESE were associated with an 80% decrease in testosterone levels at 3–6 months postoperatively, and the levels increased gradually to normal levels by 12–18 months.[10] Others reported a significant decline in the testosterone level in the long-term follow-up after micro-TESE.[11]

As per Donoso *et al*., cTESE has a rate of 80% intratesticular hematoma or structural changes on postoperative ultrasound, while Amer *et al*. found a rate of structural changes of 30% in patients who underwent micro-TESE.[9,12] Furthermore, during micro-TESE, deep tissue dissection, if possible, might be avoided by many andrologists due to its technical demands and long operative time that, therefore, carry more peri- and postoperative complications.[13]

Future comparison studies need to be conducted to compare superficial and deep dissection micro-TESE in terms of operative time, complication profile, and preoperative clinical factors, such as histopathology, hormonal profile, and testicular size. Such efforts will further refine the target criteria of such patients and thus provide better outcomes.

CONCLUSION

Men with NOA may require superficial and deep dissection of the testes to identify sperm. Superficial testicular dissection alone during micro-TESE could fail to reveal sperm in up to a fifth of the men with NOA. The presented findings suggest that although successful SRRs of deep TESE were higher than that of its superficial counterpart, there is no significant statistical difference. A larger body of evidence is needed to provide a higher grade of recommendation.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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