Comparison of upper, middle, and lower pole pathologic biopsies of the testes in patients with non-obstructive azoospermia

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ABSTRACT

Introduction: Infertility is a widespread global health issue with a multifactorial etiology, affecting a significant proportion of couples. Male factors, either alone or in combination with female factors, play a crucial role in contributing to infertility. Non-obstructive azoospermia (NOA), characterized by the absence of sperm in the ejaculate due to spermatogenic failure, represents one of the most severe forms of male infertility. Microdissection testicular sperm extraction (m-TESE) has emerged as a primary therapeutic approach for these patients. This study aimed to investigate histopathologic variances between different poles of the testicles in NOA patients undergoing m-TESE and to compare the results using the Johnsen testicular biopsy classification.
Methods: Forty-two consecutive NOA patients who underwent m-TESE between November 2022 and December 2023 were included in this prospective study. Data on patient demographics, perioperative variables, and postoperative outcomes were collected and analyzed. Testicular biopsies from the upper, middle, and lower poles were histopathologically examined, and the Johnsen testicular biopsy scoring system was used for comparison.
Results: Histologic evaluation revealed Sertoli cell only syndrome in nine cases (SCO), maturation arrest (MA) in 12 cases, and hypospermatogenesis (HS) in 21 cases. Pathologic findings were consistent across all poles of the testicle. Johnsen testicular biopsy scores showed similar results among patients. The success rates of sperm retrieval varied, with two of nine patients with SCO, four of 12 with MA, and 16 of 21 with HS achieving successful results.

Conclusions: Our study demonstrated consistent histopathologic patterns across different poles of the testis, emphasizing the importance of comprehensive histologic assessment for predicting sperm retrieval success. As a result of our study, we found that the upper middle and lower poles of the testis were similar in terms of histologic and Johnsen testicular biopsy scoring system. Future research should focus on refining histopathologic classification systems and further optimizing surgical techniques to enhance outcomes in this patient population.

INTRODUCTİON

Infertility continues to affect 15% of couples worldwide, with approximately 50% of cases attributed to male factors alone or in association with female factors [1]. Non-obstructive azoospermia (NOA), defined as the absence of sperm in the ejaculate due to failure of spermatogenesis, is the most severe form of male infertility. Surgical procedures, such as the extraction of testicular sperm under an operating microscope (m-TESE), have been the primary treatment for these patients [2]. Sperm retrieval success could be increased from 46% using an operating microscope to identify potential seminiferous tubules containing sperm, a technique known as micro-TESE [3, 4]. This method offers a clearer view of the testicular parenchyma, facilitating the identification of enlarged seminiferous tubules more likely to produce sperm. Furthermore, the extraction of smaller fragments simplifies embryologists' specimen processing and sperm search, while also reducing testicular damage and the risk of testosterone deficiency [5, 6]. Despite advancements in surgical techniques such as micro-TESE, the success of sperm retrieval remains inconsistent, particularly in cases with severe testicular damage or unfavorable histopathological patterns. There is limited understanding of whether specific regions of the testes yield higher success rates, and studies comparing histopathological characteristics across different testicular regions are scarce. This gap highlights the need for targeted approaches to optimize outcomes for patients with NOA.

The success rates of micro-TESE are largely dependent on the predominant testicular histopathological phenotype. Histological patterns associated with NOA include Sertoli cell-only syndrome (SCOS), maturation arrest (MA), and hypospermatogenesis (HS). In this study, our objective was to investigate the histopathological differences between the upper, middle, and lower poles of the testes. In addition to histopathological results, the study was also compared using the Johnsen testicular biopsy classification. Thus, we sought to determine whether focusing on one of these three poles during micro-TESE could yield significant differences in outcomes.

METHODS

This study was approved by the local ethics committee and involved 42 consecutive patients who underwent m-TESE for non-obstructive azoospermia (NOA) at our institution, performed by the same surgical team, between November 2022 and December 2023. Data were collected and analyzed prospectively. The patients were referred to our fertility clinic for sperm retrieval. Azoospermia was confirmed when two semen samples, after

centrifugation and examination at 400x magnification with a microscope, showed the absence of sperm, following the guidelines of the World Health Organization guidelines [7]. The sample size was not calculated in advance, as all eligible patients who met the inclusion criteria within the study period were consecutively included. This approach ensured that the sample was representative of our patient population and minimized selection bias. Demographic data of the patients and perioperative and postoperative variables, such as age, testicle size, levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, pathological results, and genetic findings and complications, were recorded. All patients underwent Y-chromosome microdeletions test, with none showing total azoospermia factor AZF a or AZF b deletions. Patients with a history of previous testicular sperm extraction surgeries were excluded from the study. The results of biopsies taken from different poles of the testes were compared using the Johnsen testicular biopsy scoring system.

Surgical technique

The surgical procedure was performed under intravenous sedation (IV) and local anesthesia, and all procedures were performed by the same surgeon. Before proceeding with micro-TESE, patients underwent a reevaluation of testicular volume. The selection of the testicular side for biopsy was based on the clinical history and the assessment of the testicular volume by physical examination and scrotal ultrasound. A longitudinal incision was made along the median raphe of the scrotum and then the testes were delivered through the incision. The exposed testicular parenchyma was examined under an operating microscope at 10x magnification to identify dilated seminiferous tubules. Following identification, tissue fragments were carefully transferred to a sterile dish containing sperm medium. These tissue fragments were promptly transported to an IVF laboratory where they were dissected and examined by an embryologist under high-power microscopy to confirm the presence of motile or nonmotile sperm.

Pathological examination was carried out by randomly biopsy of the upper, middle and lower poles of the testicle following the micro-TESE procedure (Figure 1),(Figure 2). Upon completion of the procedure, the incision in the albuginea was closed using a VICRYL 5/0 running suture. Bouin's solution was utilized for transferring testicular tissues. Histopathological analysis of testicular fragments was carried out by the same pathologist, with the tissues stained using hematoxylin and eosin.

The Johnsen score is a well-established histological grading system that evaluates the arrangement and maturation of germ cells within seminiferous tubules on a scale from 1 to 10, where:

- 1: Absence of germ cells in the seminiferous tubules.
- 2: Presence of Sertoli cells only.
- 3: Presence of spermatogonia without further differentiation.
- 4–7: Progressive stages of spermatogenesis, including primary spermatocytes and early spermatids.
- 8–10: Advanced stages of spermatogenesis, culminating in the presence of fully formed mature spermatozoa.

In our study, biopsy samples from the upper, middle, and lower poles of the testes were independently scored using this system by an experienced pathologist to ensure consistency and reliability. Our findings revealed no statistically significant differences in Johnsen scores between the three regions, further supporting the conclusion that spermatogenesis is evenly distributed across testicular poles in cases of non-obstructive azoospermia.
Complications observed during the study were systematically recorded and categorized. Minor complications included mild testicular pain in 12% of patients and non-severe bruising in 8%, while no major complications were reported. Limitations in data collection regarding rare or delayed complications may have impacted the comprehensiveness of our findings.

Statistical analysis
Statistical analysis was performed using SPSS software (version 16.0). Differences between groups of patients in medians for quantitative variables and distributions for categorical variables were assessed using Kruskal-Wallis one-way analysis of variance (ANOVA). A significance level of $p < 0.05$ (two-sided) was considered statistically significant.

RESULTS

Among the 42 men included in the study, the mean age was 33.2 ± 6.16 years. The mean level of follicle-stimulating hormone (FSH) was 25.9 ± 15.10 IU/L, the mean level of luteinizing hormone (LH) was 12.5 ± 9.40 IU/L, the mean testosterone level was 1.5 ± 0.6 ng/mL, and the mean testicular volume was 2.3 ± 0.7 mL (Table 1).

Histological evaluation of the specimens of these 42 patients revealed nine cases of Sertoli cell-only syndrome, 12 cases of maturation arrest, and 21 cases of hypospermatogenesis. Pathological findings were consistent across all three poles of the testes in all patients. When the Jhonsen testicular biopsy scores were compared, the results of the patients were similar biopsy scores (Table 2) .In terms of sperm retrieval success, microdissection testicular sperm extraction (m-TESE) resulted in sperm being successfully retrieved in 2 out of 9 patients with SCOS (22%), 4 out of 12 patients with MA (33%), and 16 out of 21 patients with HS (76%)(Table 3).

Furthermore, no serious intraoperative or postoperative complications were encountered in our study. Minor complications, including transient scrotal swelling, were noted in a few patients and resolved without the need for surgical intervention. In addition, mild testicular pain and non-severe bruising (ecchymosis) were observed in some patients, both of which subsided within a few days. These minor complications are typical following testicular biopsy procedures and are generally self-limiting.

Potential complications associated with micro-TESE, as described in the literature, can include hematoma formation, infection, testicular atrophy, and damage to the blood vessels or nerves. In rare cases, more serious complications such as impaired testicular function or long-term testosterone deficiency may occur. However, in our series, no patients experienced significant hematoma, infection, or other serious complications. The overall safety profile of the procedure in this cohort was favorable, highlighting the importance of careful surgical technique and postoperative management to minimize risks.

DİSCUSSİON

Testicular biopsy and histopathological examination play a crucial role in the diagnosis and treatment of male infertility, particularly in cases of non-obstructive azoospermia (NOA). Testicular biopsy and histopathological examination are pivotal in diagnosing and managing male infertility, especially in NOA cases [8]. However, the 2024 EAU guidelines indicate that there are no definitive predictors of sperm retrieval success prior to testicular sperm extraction (TESE), though histology plays a role in the evaluation process. Histological evaluation provides valuable information on testicular architecture, spermatogenesis status, and potential treatment outcomes [9, 10]. The presence of heterogeneous histologies complicates the surgical approach during m-TESE procedures. Surgeons must navigate through varying histological landscapes within testicular tissue to identify areas with spermatogenesis with precision. This requires a thorough histopathological examination of the extracted tissue to delineate regions with potential sperm production [11]. The heterogeneous nature of histology underscores the importance of precise tissue sampling and microdissection techniques to maximize sperm retrieval rates [12]. The discussion of minimizing risks associated with testicular biopsy in the results section has been incorporated here, as it aligns more closely with considerations of surgical challenges and outcomes. Several research studies have suggested that the m-TESE technique should become the standard in the treatment of patients with NOA that has significantly improved SRR, ranging between 40% and 60% [3, 13]. Many studies demonstrate successful sperm retrieval in a considerable proportion of patients with histopathological patterns of MA and HS histopathological patterns [14]. However, patients with SCO exhibit more variable outcomes, with SRR ranging widely due to the homogeneous nature of SCO and limited areas of sperm production areas within the testis [15, 16].

While m-TESE is generally considered safe and effective, it is not without potential complications. Testicular damage, hematoma formation, and transient scrotal swelling are known complications of this procedure. One of the primary complications associated with MicroTESE is testicular damage, which may result from surgical manipulation of delicate testicular tissue. Although MicroTESE involves meticulous dissection under magnification to minimize tissue trauma, inadvertent injury to blood vessels, nerves, or adjacent structures can occur. Testicular damage can manifest as hematoma formation, ischemia, or impaired testicular function after the operation. Several studies have reported the incidence of postoperative complications following MicroTESE procedures [15]. A low rate of surgical complications, including hematoma formation and transient scrotal swelling, was documented. However, it is essential to acknowledge that complications can vary depending on surgical technique, surgeon experience, and patient-specific factors [17]. In our study, no serious complications that required intervention were observed in any of our patients. Our findings support the notion that m-TESE remains the optimal approach for NOA treatment, balancing safety with high success rates for sperm retrieval.

In this study, we observed that histopathological patterns, such as Sertoli cell-only syndrome (SCO), maturation arrest (MA), and hypospermatogenesis (HS), were consistent across all poles of the testes in the examined patients. This finding aligns with previous

research that highlights the importance of comprehensive histological assessment in determining sperm retrieval success rates [2].

In light of our findings, we propose an algorithmic approach for the role of testicular biopsy in NOA management. This involves systematic sampling across testicular poles to confirm histological consistency and guide targeted m-TESE strategies.

Johnsen's scoring system (JS) categorizes testicular biopsy findings based on the arrangement of germ cells within seminiferous tubules, ranging from 1 (no germ cells) to 10 (complete spermatogenesis). This scoring system has immense clinical relevance, providing prognostic information on the likelihood of successful sperm retrieval and guiding treatment decisions for infertile men. Validation studies have underscored the predictive value of JSS in evaluating sperm retrieval rates and fertility outcomes. Smith et al. demonstrated a significant association between higher JS and increased sperm retrieval rates in men undergoing m-TESE procedures [18]. Histopathological classification, such as that serves as a valuable tool to predict sperm retrieval outcomes. This highlights the importance of targeted biopsy strategies based on histopathological findings to optimize sperm retrieval success [5]. In our study, we found that the Johnsen score was similar in different parts of the testes, and the upper middle and lower poles of the testes were not superior to each other in terms of sperm finding.

Our results underscore the importance of a comprehensive approach to testicular biopsy and histopathological evaluation in the treatment of NOA. By accurately identifying histological patterns and utilizing advanced techniques like m-TESE, clinicians can tailor treatment strategies to maximize sperm retrieval success while minimizing patient morbidity . Based on our clinical experience and findings, we propose an algorithmic approach to testicular biopsy during m-TESE in NOA patients. Initially, histopathological evaluation using the Johnsen scoring system should guide the identification of testicular regions most likely to yield sperm. In cases where no significant histopathological variability is observed across testicular poles, a comprehensive microdissection approach targeting multiple regions may enhance the likelihood of successful sperm retrieval. Incorporating advanced imaging techniques and real-time pathology assessments could further refine this algorithm, reducing tissue damage while maximizing outcomes.

Our study has several limitations. First, the relatively small sample size (42 patients) limits the generalizability of our findings. Larger, multicenter studies are needed to confirm the consistency of histopathological patterns across different testicular poles in NOA patients. Second, the observational nature of our study precludes the establishment of causal relationships between testicular histopathology and sperm retrieval success. Future randomized controlled trials could provide more definitive insights. Third, although all surgeries were performed by the same experienced surgeon, subtle variations in technique or intraoperative decision-making could introduce bias. Standardizing surgical protocols across multiple centers would mitigate this concern. Lastly, our study did not include detailed genetic or molecular analyses, which could provide a more comprehensive understanding of the underlying mechanisms of NOA and further stratify patients by prognosis. Future

research should focus on integrating genetic, molecular, and histopathological data to optimize treatment strategies and improve outcomes for this challenging patient population.

CONCLUSIONS

This study highlights the importance of comprehensive histopathological evaluation and targeted biopsy strategies in the treatment of non-obstructive azoospermia (NOA). Our findings demonstrate that histopathological patterns, such as Sertoli cell-only syndrome (SCOS), maturation arrest (MA), and hypospermatogenesis (HS), are consistently distributed across the upper, middle, and lower poles of the testes. Notably, no single pole (upper, middle, or lower) offered a distinct advantage in terms of sperm retrieval. Therefore, to optimize sperm retrieval success, it is crucial to evaluate the entire testis rather than focusing on a specific region. This comprehensive approach not only enhances sperm retrieval outcomes but also ensures that no potentially productive areas of the testis are overlooked. These findings contribute valuable insight into maximizing the effectiveness of micro-TESE procedures, supporting the continued refinement of surgical techniques to improve male infertility treatment outcomes.

REFERENCES

1. Irvine DS. Epidemiology and aetiology of male infertility. *Hum Reprod* 1998;13:33-44. https://doi.org/10.1093/humrep/13.suppl_1.33
2. Devroey P, et al. Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. *Hum Reprod* 1995;10:1457-60. https://doi.org/10.1093/humrep/10.6.1457
3. Achermann AP, Pereira TA, Esteves SC. Microdissection testicular sperm extraction (micro-TESE) in men with infertility due to non-obstructive azoospermia: Summary of current literature. *Int Urol Nephrol* 2021;53:2193-210. https://doi.org/10.1007/s11255-021-02979-4
4. Schlegel PN. Testicular sperm extraction: Microdissection improves sperm yield with minimal tissue excision. *Hum Reprod* 1999;14:131-5. https://doi.org/10.1093/humrep/14.1.131
5. Dabaja AA, Schlegel PN. Microdissection testicular sperm extraction: An update. *Asian J Androl* 2013;15:35. https://doi.org/10.1038/aja.2012.141
6. Esteves SC, Verza S. PESA/TESA/TESE sperm processing. *Pract Man In Vitro Fertil* 2012;207-20. https://doi.org/10.1007/978-1-4419-1780-5_24
7. Esteves SC. Clinical relevance of routine semen analysis and controversies surrounding the 2010 World Health Organization criteria for semen examination. *Int Braz J Urol* 2014;40:433-53. https://doi.org/10.1590/S1677-5538.IBJU.2014.04.02
8. Irvine DS. Epidemiology and aetiology of male infertility. *Hum Reprod* 1998;13:33-44. https://doi.org/10.1093/humrep/13.suppl_1.33
9. Haimov-Kochman R, et al. The value of repeat testicular sperm retrieval in azoospermic men. *Fertil Steril* 2009;91:1401-3. https://doi.org/10.1016/j.fertnstert.2008.04.066
10. Colpi GM, et al. Sperm retrieval for intra-cytoplasmic sperm injection in non-obstructive azoospermia. *Minerva Urol Nefrol* 2005;57:99-107.
11. Schlegel PN, Li PS. Microdissection TESE: Sperm retrieval in non-obstructive azoospermia. *Hum Reprod Update* 1998;4:439. https://doi.org/10.1093/humupd/4.4.439
12. Esteves SC, Miyaoka R, Agarwal A. An update on the clinical assessment of the infertile male. *Clinics (Sao Paulo)* 2011;66:691-700. https://doi.org/10.1590/S1807-59322011000400026
13. Tsujimura A, et al. Conventional multiple or microdissection testicular sperm extraction: A comparative study. *Hum Reprod* 2002;17:2924-9. https://doi.org/10.1093/humrep/17.11.2924
14. Ramasamy R, Schlegel PN. Microdissection testicular sperm extraction: Effect of prior biopsy on success of sperm retrieval. *J Urol* 2007;177:1447-9. https://doi.org/10.1016/j.juro.2006.11.039
15. Schlegel PN. Testicular sperm extraction: Microdissection improves sperm yield with minimal tissue excision. *Hum Reprod* 1999;14:131-5. https://doi.org/10.1093/humrep/14.1.131
16. Anniballo R, et al. Criteria predicting the absence of spermatozoa in the Sertoli cell-only syndrome can be used to improve success rates of sperm retrieval. *Hum Reprod* 2000;15:2269-77. https://doi.org/10.1093/humrep/15.11.2269
17. Amer M, et al. Prospective comparative study between microsurgical and conventional testicular sperm extraction in non-obstructive azoospermia: Followup by serial ultrasound examinations. *Hum Reprod* 2000;15:653-6. https://doi.org/10.1093/humrep/15.3.653

18. Smith JF, et al. Sexual, marital, and social impact of a man's perceived infertility diagnosis. *J Sex Med* 2009;6:2505-15. https://doi.org/10.1111/j.1743-6109.2009.01383.x

FIGURES AND TABLES

Figure 1. Random biopsy of the upper middle and lower poles of the testis.

Figure 2. Upper middle and lower pole image of a patient with Sertoli cell-only syndrome pathology. Sections were stained with hematoxylin and eosin (H&E) and examined under light microscopy at 10x magnification.

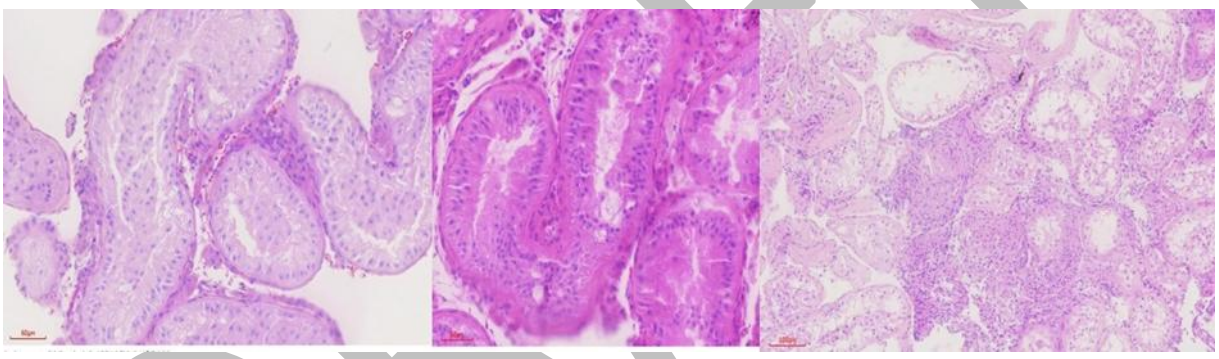

Table 1. Baseline characteristics of patients (n=42)	
Parameter	**Mean ± SD**
Age (years)	33.2±6.16
Follicle-stimulating hormone (IU/L)	25.9±15.10
Luteinizing hormone (IU/L)	12.5±9.40
Testosterone (ng/mL)	1.5±0.6
Testicular volume (mL)	2,3±0.7

SD: standard deviation.

Table 2. Comparison of Johnsen scores across the upper, middle, and lower poles of the testis in different histopathologic patterns				
Histopathology	**Upper pole (n)**	**Middle pole (n)**	**Lower pole (n)**	**p**
Sertoli cell only syndrome	1.33±0.5	1.55±0.42	1.35±0.52	0.64
Maturation arrest	5.16±1.69	5.16±1.52	5.33±1.66	0.95
Hypospermatogenesis	7.76±0.7	7.71±0.78	7.80±0.60	0.90

Johnsen score (mean ± standard deviation).

Table 3. Histopathologic results of upper, middle, and lower pole testicular tissues and sperm retrieval rates				
Histopathology	**Upper pole (n)**	**Middle pole (n)**	**Lower pole (n)**	**Successful sperm retrieval (rate (%)**
Sertoli cell only syndrome	9	9	9	2 (22%)
Maturation arrest	12	12	12	4 (33%)
Hypospermatogenesis	21	21	21	16 (76%)