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 affects 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 by 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 histopathologic patterns. There is limited understanding of whether specific regions of the testes yield higher success rates, and studies comparing histopathologic 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 m-TESE are largely dependent on the predominant testicular histopathologic phenotype. Histologic 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 histopathologic differences between the upper, middle, and lower poles of the testes. In addition to histopathologic results, the Johnsen testicular biopsy scoring system was used for comparison. Thus, we sought to determine whether focusing on one of these three poles during m-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 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.[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.

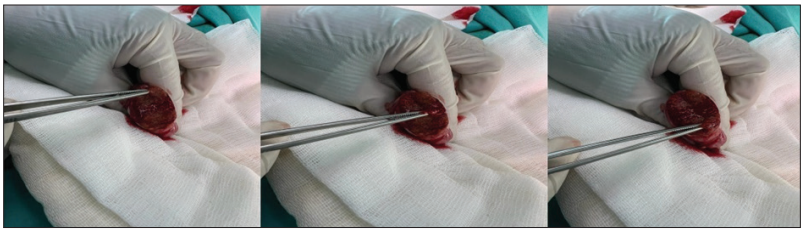

Figure 1. Random biopsy of the upper middle and lower poles of the testis.

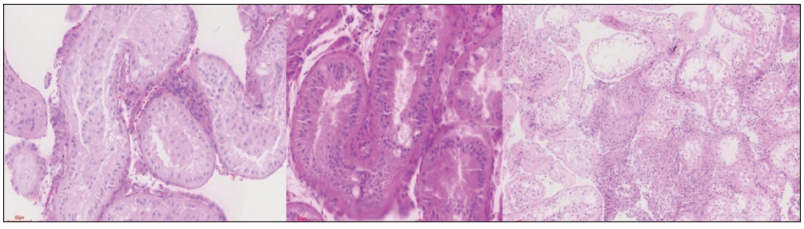

Figure 2. Upper middle and lower pole image of a patient with Sertoli cell-only syndrome pathology. Sections were stained with hematoxylin and eosin (H&E) and examined under light microscopy at 10x magnification.

Demographic data of the patients and perioperative and postoperative variables such as age; testicle size; levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone; pathologic results; and genetic findings and complications were recorded. All patients underwent Y-chromosome microdeletions test, with none showing total azoospermia factor AZFa or AZFb 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 (IV) sedation and local anesthesia all by the same surgeon. Before proceeding with m-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 non-motile sperm.

Pathologic examination was carried out by random biopsy of the upper, middle, and lower poles of the testicle following the m-TESE procedure (Figures 1, 2). Upon completion of the procedure, the incision in the albuginea was closed using a VICRYL 5/0 running suture. Bouin's solution was used for transferring testicular tissues. Histopathologic 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 histologic grading system that evaluates the arrangement and maturation of germ cells within seminiferous tubules on a scale of 1–10, 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.

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 FSH was 25.9±15.10 IU/L, the mean level of 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).

Histologic evaluation of the specimens of these 42 patients revealed nine cases of SCO, 12 cases of MA, and 21 cases of HS. Pathologic findings were consistent across all three poles of the testes in all patients. When the Johnsen testicular biopsy scores were compared, the results were similar (Table 2). In terms of sperm retrieval success, m-TESE resulted in sperm being successfully retrieved in 2/9 patients with SCOS (22%), 4/12 patients with MA (33%), and 16/21 patients with HS (76%) (Table 3).

Potential complications associated with m-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. 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 (in 12% of patients) and non-severe bruising (in 8% of patients) were observed, both of which subsided within a few days. These minor complications are typical following testicular biopsy procedures and are generally self-limiting.

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.

DISCUSSION

Testicular biopsy and histopathologic examination play a crucial role in the diagnosis and treatment of male infertility, particularly in cases of NOA. Testicular biopsy and histopathologic examination are pivotal in diagnosing and managing male infertility, especially in NOA cases;[8] however, the 2024 European Association of Urology guideline indicates that there are no definitive predictors of sperm retrieval success prior to TESE, although histology plays a role in the evaluation process.

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%)

Histologic 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 histologic landscapes within testicular tissue to precisely identify areas with spermatogenesis. This requires a thorough histopathologic examination of the extracted tissue to delineate regions with potential sperm production and maximize sperm retrieval rates.[11,12]

Several research studies have suggested that the m-TESE technique should become the standard in the treatment of patients with NOA, as it has improved sperm retrieval rates 40–60%.[3,13] In particular, the literature has demonstrated successful sperm retrieval in a considerable proportion of patients with MA and HS histopathologic patterns;[14] however, patients with SCO exhibit more variable outcomes, with sperm retrieval rates ranging widely due to the homogeneous nature of SCO and limited areas of sperm production 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 m-TESE is testicular damage, which may result from surgical manipulation of delicate testicular tissue. Although m-TESE 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 m-TESE.[15] 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 requiring 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.

We also observed that histopathologic patterns, such as SCO, MA, and 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 histologic assessment in determining sperm retrieval success rates.[2]

The Johnsen scoring system 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 Johnsen scoring system in evaluating sperm retrieval rates and fertility outcomes. Smith et al demonstrated a significant association between higher Johnsen score and increased sperm retrieval rates in men undergoing m-TESE procedures.[18] 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.

In light of our findings, we propose an algorithmic approach for the role of testicular biopsy in NOA management. Initially, histopathologic evaluation using the Johnsen scoring system should guide the identification of testicular regions most likely to yield sperm. In cases where no significant histopathologic 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.

Limitations

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 histopathologic 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 histopathologic data to optimize treatment strategies and improve outcomes for this challenging patient population.

CONCLUSIONS

This study highlights the importance of comprehensive histopathologic evaluation and targeted biopsy strategies in the treatment of NOA. Our findings demonstrate that histopathologic patterns, such as SCO, MA, and 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 m-TESE procedures, supporting the continued refinement of surgical techniques to improve male infertility treatment outcomes.

COMPETING INTERESTS: The authors do not report any competing personal or financial interests related to this work.

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