

Delayed Detection of Sperm in the Ejaculate Following Failed Microdissection Testicular Sperm Extraction in a Man With Azoospermia: A Case Report

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ABSTRACT

Introduction: Non-obstructive azoospermia (NOA) affects 10%–15% of infertile males, posing a significant challenge in fertility management. Microdissection testicular sperm extraction (mTESE) is regarded as the gold standard for sperm retrieval for NOA. However, even when mTESE fails to retrieve sperm, unexpected postoperative findings may still occur. **Case:** A 31-year-old male with primary infertility and azoospermia underwent two surgical sperm retrieval procedures: percutaneous epididymal sperm aspiration (PESA) and mTESE. Hormonal and genetic evaluations revealed no abnormalities, but no sperm were retrieved during either procedure. Remarkably, six days after surgery, a self-ejaculated sample yielded five motile spermatozoa, which were immediately cryopreserved for intracytoplasmic sperm injection. Histopathology indicated moderate spermatogenesis with Johnsen scores of 6.0 and 7.0 for the right and left testes, respectively, highlighting residual spermatogenic activity. **Discussion:** The postoperative appearance of spermatozoa may reflect focal or intermittent spermatogenesis that was not detected during surgical sampling. The histopathological findings support the presence of limited residual sperm production despite unsuccessful PESA and mTESE. **Conclusion:** The detection of motile spermatozoa after failed mTESE highlights the importance of postoperative for follow-up, repeated semen analysis, and timely cryopreservation in the fertility management of patients with NOA.

Keywords: Azoospermia, case report, microdissection testicular sperm extraction, mTESE, non-obstructive azoospermia, sperm retrieval.



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INTRODUCTION

Azoospermia is defined as the absence of sperm in natural ejaculation. Azoospermia affects almost 10%–15% of infertile males.¹ Non-obstructive azoospermia (NOA) is found in 60% of the population.¹ In the past, patients with azoospermia were considered infertile. With advancements in medical knowledge, it is now recognised that some causes of azoospermia may be reversible. Consequently, all patients with azoospermia require thorough evaluation to identify the underlying cause and ensure appropriate management.¹

Microdissection testicular sperm extraction (mTESE) is considered a gold-standard surgical procedure (Figure 1) for sperm retrieval in patients with non-obstructive

azoospermia (NOA).² This technique involves examining testicular tissue under a microscope to identify blood vessels and sites of sperm production in men with NOA. The complication rate of mTESE is exceptionally low, particularly when performed by experienced surgeons.³ Several factors influence the success of mTESE, including age, testicular volume, levels of follicle-stimulating hormone and luteinizing hormone, hypospermatogenesis, sertoli-cell-only syndrome, and maturation arrest.⁴ The success rate of mTESE in retrieving sperm ranges from 22% to 77%.⁵ However, in certain cases, mTESE may be unsuccessful due to a variety of factors.

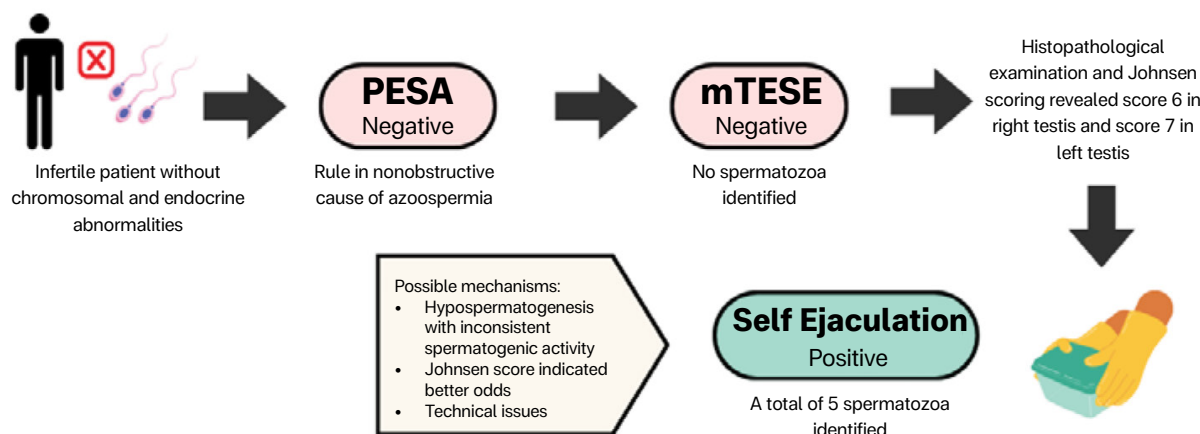
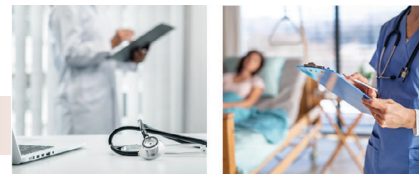
CASE

A 31-year-old NOA patient presents with

primary infertility and is diagnosed with azoospermia, defined as a complete absence of spermatozoa in the ejaculate. To prepare for assisted reproductive technology (ART), the patient underwent two consecutive procedures: Percutaneous Epididymal Sperm Aspiration (PESA) and Microdissection Testicular Sperm Extraction (mTESE) on the right and left testicles. After the procedures, the patient recovered at home. On the sixth day post-operation, he was instructed to perform self-ejaculation for a follow-up assessment at our fertility clinic.

The patient's medical history revealed no significant health issues. Initial evaluations included a semen analysis, hormone assessment, and genetic testing. The semen analysis confirmed azoospermia,

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Figure 1. A diagram illustrating the patient's procedures and results.

Abbreviations: PESA: Percutaneous epididymal sperm aspiration; mTESE: microdissection testicular sperm extraction.

while hormonal evaluation revealed normal testosterone level of 3.2 ng/mL (normal range: 2.49–8.36), follicle-stimulating hormone (FSH) of 9.65 mIU/mL (1.5–12.4), and luteinizing hormone (LH) of 5.82 mIU/mL (1.70–8.60), suggesting that the issue was not due to an endocrine abnormality.⁶ Genetic analysis showed no chromosomal abnormalities, as the Y Chromosome Microdeletion test confirmed that there are no microdeletions, ruling out genetic causes of azoospermia.

Given these results, the patient's urologist recommended surgical sperm retrieval to explore the possibility of locating viable sperm for assisted reproductive technology (ART). PESA was initially performed to aspirate sperm directly from the epididymis. Unfortunately, this attempt did not yield any spermatozoa, suggesting either a failure of sperm production or an obstruction preventing sperm from reaching the epididymis.

Following the unsuccessful PESA, mTESE was carried out. We performed mTESE under a microscope to identify seminiferous tubules that appear larger or fuller, as these are more likely to contain sperm in both testes.

No sperm was identified under microscope examination (**Figure 2A** and **2B**). Following this, a histopathological examination of testicular tissue was performed, focusing on the Johnson score. The right testis was assigned a Johnson score of 6.0, indicating that spermatogenesis had progressed to the spermatocyte stage with some spermatid formation but no mature spermatozoa; the left testis had a Johnson score of 7.0, where spermatogenesis reached the spermatid stage, yet still did not result in mature spermatozoa.⁷ These findings underscore the limited spermatogenic activity and provide insight into the patient's ability to produce sperm, even in a significantly reduced capacity.

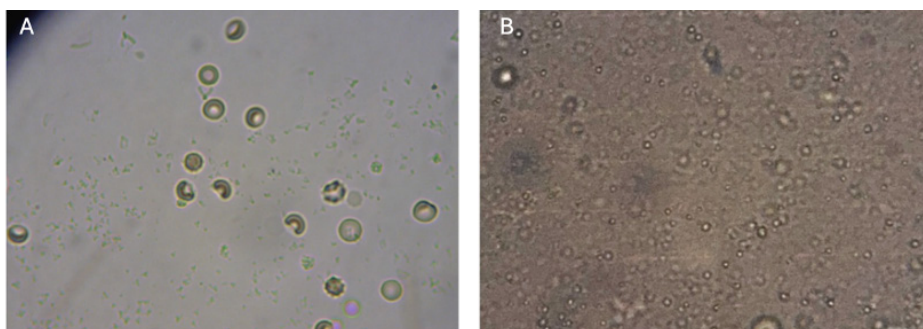
Microscopic examination of the retrieved specimens (PESA/mTESE) revealed no spermatozoa. The sample contained mostly erythrocytes, occasional lymphocytes, and cellular debris.

Six days after the procedures, the self-ejaculation sample was collected. Analysis revealed the presence of five spermatozoa in the ejaculate, indicating limited, but detectable sperm production. Although extremely low in number, the sperm identified were viable for use in an ICSI program. The sperm were immediately cryopreserved in preparation for ovarian stimulation of the patient's wife.

DISCUSSION

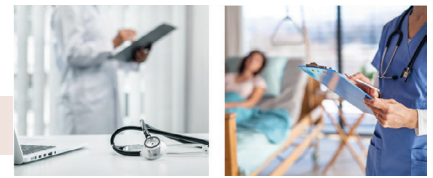
This case presents an intriguing scenario of a NOA case in which motile spermatozoa appeared spontaneously in the ejaculate six days after failed mTESE. These findings emphasize the complexity of NOA and the need to integrate comprehensive follow-up strategies in fertility management.

Focal spermatogenesis, as demonstrated in this case, highlights the localized nature of sperm production within seminiferous tubules. Histological examination revealed Johnson scores of 6 and 7, consistent with spermatogenic arrest and incomplete spermatogenesis. These scores indicate active germ cell layers capable of producing spermatids but failing to progress to mature sperm. Such focal activity is common in NOA and may evade detection during mTESE



*Photo documentation by Ponco Birowo.

Figure 2. Microscope examination. (A) Percutaneous Epididymal Sperm Aspiration results (PESA); (B) Microdissection Testicular Sperm Extraction (mTESE).



because functional tubules are patchily distributed.^{8,9} Localized microenvironments within the testes may protect certain regions, allowing limited spermatogenic activity despite broader testicular dysfunction. This underscores the importance of meticulous and widespread sampling during mTESE to maximize the likelihood of detecting viable sperm.⁸

The patient's hormonal profile showed FSH levels of 9.65 mIU/mL and LH levels of 5.82 mIU/mL, both within normal ranges. These findings are reassuring, as elevated FSH is often associated with severe testicular damage and poorer outcomes. In contrast, normal levels of FSH and LH, particularly in combination with moderate testicular volume, suggest a higher probability of successful sperm retrieval.^{9,10} Hypospermatogenesis, as evidenced by histological findings in this case, further supports the potential for focal spermatogenesis. Studies indicate that hypospermatogenesis is the most favourable histopathological pattern for sperm retrieval, with success rates often exceeding 88.2%.¹¹ The testosterone-to-LH ratio, though not analyzed in this case, has been proposed as an additional marker for predicting spermatogenic activity.¹²

This case also underscores the technical challenges inherent to mTESE. Despite its precision, mTESE may fail to detect focal spermatogenesis due to insufficient sampling or operator error. Additionally, the sporadic nature of sperm production in NOA poses significant challenges, as viable sperm may be present but not collected during the procedure. The histological findings of Johnsen scores 6 and 7 suggest limited but present spermatogenesis, further emphasizing the importance of optimizing sampling strategies.^{9,13} The spontaneous appearance of sperm in the ejaculate post-mTESE raises questions about the role of delayed spermatogenesis or residual sperm release. While such findings are rare, they offer an important reminder to include post-

operative evaluations as part of routine fertility management for NOA patients.¹⁴

In rare cases, there may be concerns about whether sperm detected in the ejaculate post-mTESE could have been externally introduced. While this scenario is uncommon, ensuring sample authenticity is critical to maintaining trust in fertility management.^{15,16}

One simple approach to detect false reporting is to compare the ejaculate volume of the post-mTESE sample with pre-mTESE samples. Typically, the introduction of external sperm is associated with an unusually high ejaculate volume, which would stand out during analysis. Another option, albeit more invasive, is to conduct DNA testing to verify the origin of the sperm. However, this method may undermine the trust relationship between the patient and the clinician, making it a less ideal approach in most cases.^{15,16} To prevent such scenarios, the most reliable method is to ensure the ejaculate sample is collected under direct supervision in a laboratory setting. Patients should be advised not to bring samples collected at home to avoid any doubts about the specimen's integrity. Such protocols not only minimize the risk of false reporting but also reinforce the transparency and trustworthiness of the fertility management process.^{17,18}

The discovery of motile sperm in post-operative ejaculation demonstrates the importance of follow-up evaluations. These findings allow for cryopreservation of sperm, providing patients with an opportunity for assisted reproductive techniques like ICSI. While fresh sperm is often associated with higher fertilization and live birth rates compared to cryopreserved sperm, the ability to secure viable gametes without additional invasive procedures represents a significant clinical advantage.^{17,18}

However, cases like this are exceedingly rare. Patients undergoing mTESE must be thoroughly counselled beforehand to

understand that mTESE is typically considered the last resort in sperm retrieval for NOA. False hope should not be given, as the likelihood of spontaneous sperm appearance in the ejaculate post-mTESE is exceptionally low. Clear communication is essential to ensure that patients have realistic expectations regarding the outcomes of mTESE and the subsequent chances of achieving fertility.^{17,19}

CONCLUSION

This case underscores the importance of recognising focal spermatogenesis as a dynamic process that may be missed during initial sperm retrieval attempts. Incorporating detailed histological evaluations, hormonal profiling, and strategic follow-up assessments into fertility protocols is essential for optimising outcomes in NOA patients. Further research is needed to enhance our understanding of delayed sperm appearance and improve the efficacy of sperm retrieval techniques.

Consent for Publication

Written informed consent for publication of this case report and any accompanying images was obtained from the patient.

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Author Contributions

MAT, ESS, FA, FA, and AA contributed to the study design, data collection, data analysis, and preparation of the draft manuscript. WA, NR, and PB contributed to data collection, manuscript review, and editing. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

Declaration on the Use of Artificial Intelligence (AI)

The authors used ChatGPT (OpenAI) to assist with language editing, grammar correction, and sentence refinement during the preparation of this manuscript. The final manuscript was reviewed and approved by all authors, who take full responsibility for its content.

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