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Can We Predict the Outcome of Micro Testicular Sperm Extraction in Non-Obstructive Azoospermia From Preoperative Hormonal Profile, Testicular Volume, and Patients Health Factors: A Retrospective Cross-Sectional Study

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Abstract

Infertility is characterized by the inability to conceive even after engaging in regular unprotected sexual intercourse for a period of 12 months or longer. Azoospermia affects around 1% of men. Approximately 60% of men diagnosed with azoospermia will have non-obstructive azoospermia (NOA). The main aim of this study is to investigate the potential relationship between preoperative hormonal profiles, testicular volume, and patient health factors with microdissection testicular sperm extraction (micro-TESE) outcomes in individuals with NOA. A retrospective analysis of 152 patients who underwent a micro-TESE operation for NOA at our center from January 2020 to December 2022 was conducted. Both groups were compared for age, follicle-stimulating hormone (FSH), luteinizing hormone (LH), total and free testosterone, testicular volume before the operation, previous TESE, smoking, and medical illnesses. A relationship is considered significant when the p value is less than .05. A total of 152 NOA patients were enrolled in this study. Patients were divided into two groups: first group, in whom sperms were identified during the procedure, representing 72 (47.3%) of patients, and the second group (52.7%) of patients, in whom no sperms were found. Results reveal that free testosterone level, total testosterone level, smoking, and previous TESE operation are significantly related to positive surgical results (p value $< .05$). Our findings suggest that preoperative total and free testosterone levels, smoking status, and previous micro-TESE operation may significantly affect the outcomes of micro-TESE.

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Introduction

Infertility is a medical condition that affects the male or female reproductive system, characterized by the inability to conceive even after engaging in regular unprotected sexual intercourse for a period of 12 months or longer (World Health Organization [WHO], 2024). Approximately 15% of the general population experiences challenges with fertility. A condition characterized by the complete absence of sperm in two distinct centrifuged semen samples is referred to as azoospermia, which represents the most severe form of male infertility. Azoospermia affects around 1% of men and accounts for approximately 10% to 15% of all cases of male infertility. In the male reproductive system, difficulties with fertility often arise from issues related to the release of semen, insufficient or absent sperm production, or abnormalities in the shape (morphology) and movement (motility) of sperm. These factors are the primary causes of male infertility (Aziz, 2013; Esteves et al., 2011).

The majority, approximately 60%, of men diagnosed with azoospermia will have non-obstructive azoospermia (NOA), while the remaining will have obstructive azoospermia, in which sperms normally produced in testes but there is an obstruction in the pathways to transfer sperms outside the body. NOA is typically caused by severe abnormalities in spermatogenesis, often resulting from primary testicular failure or dysfunction. It can also result from dysfunction in the pituitary or hypothalamus. The precise underlying cause of NOA is often unknown or idiopathic. Advanced assisted reproductive techniques have proven effective in treating NOA, particularly in cases of primary testicular failure (Moon et al., 2006).

The complexities surrounding NOA are further accentuated by the variability observed in sperm retrieval rates (SRR) during microdissection testicular sperm extraction (micro-TESE). Factors such as age, serum levels of follicle-stimulating hormone (FSH), testosterone (T), and even prior varicocele repair have been identified as potential influencers of SRR (Aljubran et al., 2022). Recent literature has shed light on the nuanced relationship between FSH levels and SRR, with some studies suggesting a weak correlation, while others emphasize the critical role of bilateral testis retrieval and the diameter of seminiferous tubules (Sabudi, 2020).

A broader examination of the NOA landscape reveals a range of SRR outcomes, with rates fluctuating between 43% and 63% for men undergoing micro-TESE (Falcone et al., 2022). This variability underscores the multifactorial nature of NOA, influenced by elements such as testicular histopathology patterns, testicular volume, and the specific surgical techniques employed during TESE (Salehi et al., 2017). Genetic factors, including the presence of AZFc deletion or Klinefelter's syndrome, are also highlighted as significant determinants in sperm retrieval outcomes (Qi et al., 2021).

The profound implications of NOA on male fertility extend beyond the physiological realm, impacting the psychosocial well-being of affected individuals. As such, a comprehensive understanding of the myriad factors influencing SRR is of paramount importance. This study embarks on an in-depth exploration of the determinants of SRR in NOA patients undergoing micro-TESE. By delving into the intricacies of NOA and its associated challenges, this research aims to offer invaluable insights that could refine clinical approaches, enhance patient counseling, and ultimately foster improved reproductive outcomes.

Furthermore, the role of diagnostic biopsies in predicting SRR has been a topic of debate. While some studies have shown that a diagnostic biopsy, hormone levels, volume of the testis, and age are potential predictive factors for sperm retrieval, others have indicated that none of these parameters can precisely predict the SRR. This ambiguity in the literature underscores the need for more comprehensive studies that can provide clearer guidelines for clinicians. So, the main objective of this retrospective study is to investigate the potential relationship between preoperative hormonal profiles, testicular volume, and patient health factors with the outcomes of micro-TESE in individuals diagnosed with NOA.

Materials and Methods

Study Design & Approval

After obtaining an institutional review board approval number (IRB/2023/515), a retrospective analysis of 152 patients who underwent micro-TESE operation for NOA at our center from January 2020 to December 2022 was conducted. All the patients had

primary infertility (their wives did not have previous pregnancies). Patients were divided into two groups: the first group had positive micro-TESE (we found viable motile sperms), while the second group had negative micro-TESE (no viable sperms found).

All micro-TESE procedures were performed under general anesthesia. An incision was created along the scrotal midline, and the tunica albuginea was incised near its midportion in the least vascular area. Under an operating microscope (Karl Zeiss, Germany), dissection and examination of the testicular parenchyma were conducted at $\times$15–20 magnification. Thick and dark yellow seminiferous tubules were harvested and fragmented with a pair of sterile 1 mL syringes with attached needles into a homogeneous suspension in a dish containing G-MOPS-plus medium (Vitrolife, Vastra Frolunda, Sweden). Then, we used an inverted microscope at $\times$200 magnification to look for the presence of sperms. Successful sperm retrieval was confirmed upon sperm identification. If no sperms were detected in the specimen retrieved, closure of tunica albuginea was done and delivery of the other testicle through the same incision, then opening of tunica albuginea and searching for sperms under the microscope as in the previous testicle were performed.

Patients discharged on the same day (day case procedure). Only one patient developed scrotal hematoma postoperation, which was managed conservatively.

Both groups were compared for age, FSH, LH, total and free testosterone, testicular volume before the operation, previous TESE, smoking, and medical illnesses. Participants were asked about their smoking habits, including whether they smoked daily, occasionally, or rarely. A relationship is considered significant when the p value is less than .05.

Inclusion and Exclusion Criteria

All patients with primary infertility who underwent micro-TESE for NOA from January 2020 to December 2022 were included in the study protocol. Patients with secondary infertility, obstructive azoospermia, and patients who did not have records for hormonal profile (FSH, LH, and testosterone) or testicular volume before operation were excluded from the study. In the end, 152 patients were included in the study.

Patient Assessment

Patient age, hormonal profile (FSH, LH, total and free testosterone), testicular volume before operation, previous TESE, and medical illnesses in addition to smoking were assessed. In this study, patients who reported smoking at least one pack of cigarettes daily were classified as current smokers. Patients were divided into two groups according to micro-TESE operation findings.

Outcome Measures and Assessment Tools

The primary outcome was to assess whether we can predict micro-TESE result (SRR) from some variables before the operation like age, smoking, previous TESE, hormonal profile, and testicular volume.

Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics 25.0 software. The dependent value is the finding of sperms during micro-TESE. For independent samples, a T test was used for numerical variables (age, FSH, LH, total and free testosterone, testicular volume), while for categorical variables (previous TESE, smoking, medical illnesses), chi-square test was used. Differences were considered statistically significant at $p < .05$.

Results

A total of 152 NOA patients were enrolled in this study. Patients were divided into two groups according to the micro-TESE result: the first group, in whom sperms were identified during the procedure representing 72 (47.3%) of patients, and in the second group (52.7%) of patients, no sperms were found after exploring both testes. Different variables that may affect micro-TESE results were evaluated for each group to investigate their effect on postoperative findings. These are divided into variables related to medical history (age, smoking status, past medical illness, and previous testicular sperm extraction), variables identified by laboratory investigation (FSH, LH, total testosterone, and free testosterone), and, finally, factors detected by radiological work up (testicular volume) measured by scrotal Doppler ultrasound. The mean age of patients is 32 years with a range of 18 to 52 years old; when analyzing the medical history of patients, seven (4.6%) of them had diabetes mellitus (DM) and six (3.9%) patients were hypertensive (HTN) at the time of infertility diagnosis. As an important risk factor for male infertility, 69 (45%) patients were smokers. According to a 2014 WHO survey, approximately 54.9% of males in our country were current smokers. While these numbers are slightly out of date, they still provide an accurate picture of smoking frequency among men in the region.

Table 1. Different Variables, Percentage, Mean, Range, and Standard Deviation

Characteristic	*n*	%	*M*	Range	*SD*
Patients (*n* = 152)	152				
Age			32	18–52	6.136
Smoking	yes=69, no=83	45%			
Diabetes mellitus	7	4.60%			
Hypertension	6	3.90%			
Measures					
Testicle volume (mL)	152		13.23	4–22.4	± 3.42
FSH (IU/mL)	152		20.79	1–82	15.383
LH (IU/mL)	152		17.09	0.9–65	11.181
Total testosterone (ng/mL)	152		3.02	0–8	1.726
Free testosterone (pg/mL)	152		18.79	1–50	9.233
Previous micro-TESE trials					
Null	79	52%			
One	46	30.30%			
Two	19	13%			
Three	4	2.60%			
Four	3	2%			

Given this high percentage, the large number of smokers in our study is expected (WHO, 2014). Normally, adult testes are ovoid in shape and measure approximately 3 cm in depth, 2 to 4 cm transversely, and 3 to 5 cm longitudinally, with a volume of 12.5 to 25 mL (W. Kim et al., 2007). In our study, the mean testicular volume preoperatively was 13.23 mL with a range of 4 to 22.4 mL. All patients have hormonal profiles before operation, including FSH, LH, total testosterone, and free testosterone. Seventy-nine (52%) patients did not have previous micro-TESE, while 46 (30.3%) patients had previous one testicular exploration for sperm extraction, 19 (13%) patients had previous two testicular sperm exploration before, and eight (4.7%) underwent three or more testicular sperm extraction before.

Table 1 shows different variables, percentages, mean, range, and standard deviation.

Utilizing binary logistic regression to examine the predictive values of these variables reveals that free testosterone level (OR = 1.074, 95% confidence interval [CI] = [1.025, 1.125], $p = .003$) and total testosterone level (OR = 1.405, 95% CI = [1.039, 1.900], $p = .027$) are significantly and positively associated with positive surgical results with. Conversely, smoking is significantly and inversely associated with positive surgical outcomes (OR = 0.396, 95% CI = [0.161, 0.972], $p = .043$). Also, previous TESE operation is significantly and inversely associated with positive surgical outcomes, with OR = 0.251, 95% CI = [0.097, 0.649], $p = .004$ for the previous one TESE and OR = 0.085, 95% CI = [0.018, 0.408], $p = .002$ for previous two TESEs, while after the previous three or more TESEs, p values were not significant (p value $> .05$).

Age and past medical history were shown to have no significant impact on sperm extraction outcomes (p values of .232 and .156, respectively). Similarly, while testosterone levels were linked to sperm extraction success, other laboratory markers, such as FSH and LH, have no statistically significant relationship (p values of .077 and .466, respectively). Although the relationship between testicular volume and sperm retrieval was not statistically significant ($p = .059$), the marginal nature of the result suggests that it may have practical value. This discovery, especially given the limited sample size, warrants additional examination.

Table 2 demonstrates logistic regression results with p value and odds ratio for different variables.

Discussion

NOA is characterized by the total absence of spermatozoa in the ejaculate, even after centrifuging the semen sample and examining the pellet microscopically (Kherraf et al., 2022). In NOA, there is reduced sperm production, which is typically associated with dysfunction of the hypothalamic–pituitary–testis axis (Oduwole et al., 2021) affecting hormones like LH, FSH, and testosterone, which are essential for spermatogenesis (Spahovic et al., 2020b). Moreover, numerous karyotype abnormalities may cause impaired spermatogenesis, including Klinefelter syndrome, translocations, deletions, and Y chromosome microdeletions as well (Khabour et al., 2014; Peña et al., 2020).

Etiologies of NOA can be either genetic and congenital, like Kallman syndrome, or acquired, including pituitary tumor, radiation, extirpation, and exogenous

Table 2. Logistic Regression Results With p-Value and Odds Ratio for Different Variables

Variable	*p* value	Odds ratio	95% CI for EXP(B)
Testicle volume (mL)	.059	1.107	[0.996, 1.229]
FSH (IU/mL)	.077	1.051	[0.995, 1.111]
LH (IU/mL)	.446	0.971	[0.899, 1.048]
Free testosterone (pg/mL)	.003	1.074	[1.025, 1.125]
Total testosterone (ng/mL)	.027	1.405	[1.039, 1.900]
Smoking	.043	0.396	[0.161, 0.972]
Diabetes mellitus	.156	4.926	[0.543, 44.686]
Hypertension	.249	3.217	[0.440, 23.505]
Age	.232	0.951	[0.875, 1.033]
Previous micro-TESE	.014	—	—
Previous one micro-TESE	.004	0.251	[0.097, 0.649]
Previous two micro-TESE	.002	0.085	[0.018, 0.408]
Previous three micro-TESE	.999	0.000	0.000
Previous four micro-TESE	.994	1.010	[0.067, 15.255]
Previous five micro-TESE	1.000	0.000	0.000

Note. Utilizing binary logistic regression to examine the predictive values of these variables reveals that free testosterone level and total testosterone level are significantly (p value $< .05$) and positively associated (odds ratio > 1) with positive results. Conversely, smoking is significantly (p value $< .05$) and inversely associated (odds ratio < 1) with positive outcomes.

androgens (Flannigan et al., 2017). The causes of NOA in our study population included idiopathic cases and Klinefelter syndrome. We conducted subgroup analyses to explore differences in outcomes between these groups, although the small sample size limited the robustness of these analyses.

The impact of testosterone levels on micro-TESE outcome prediction varies, while some studies show a relationship between testosterone levels and micro-TESE results (Mehmood et al., 2019; Zarezadeh et al., 2021) as in our study which revealed that both the total and free testosterone levels are significantly and positively correlated with favorable outcomes in micro-TESE, possibly due to testosterone's role in fertility, which is stimulated by LH and then works with FSH to produce sperm, and thus testosterone level is an indicator of sperm count (Patel et al., 2019).

However, there is conflicting evidence regarding this association. For instance, a study found that the AMH/testosterone ratio is a more reliable predictor of sperm retrieval in NOA cases than testosterone levels alone (Alfano et al., 2017). Similarly, others have demonstrated that AMH levels are associated with sperm retrieval and testicular pathology in NOA (Pozzi et al., 2023, 2024), while, on the contrary, several other studies have shown that testosterone levels had no relationship (Alrabeeah et al., 2021; Enatsu et al., 2015; Spahovic et al., 2020b).

The other factor negatively affecting micro-TESE results in our study is smoking. In 2010, a study found that smokers had lower seminal zinc levels than nonsmokers, with decreases in sperm concentration, motility, and morphology, indicating that zinc concentrations could play a role (Liu et al., 2010). A male rat study found that rats exposed to oral nicotine experienced significant decreases in sperm motility and sperm count. Thus, nicotine may also play an important role in the adverse effects of smoking on fertility, independent of the toxins found in the smoke (Oyeyipo et al., 2011). However, it should be noted that this study does not precisely assess how smoking affects overall male fertility over time. Instead, we aim to find out whether smoking affects the success rate of sperm retrieval in micro-TESE procedures for men with NOA. While smoking is known to have a detrimental impact on fertility, the purpose of this study is to investigate whether it has a direct effect on the outcome of this surgical procedure.

Despite initial hypotheses suggesting a potential link between LH and FSH with micro-TESE outcomes as predictors, our analysis yielded an absence of significant association between the two variables, in contrast to previous studies (Eken & Gulec, 2018; Spahovic et al., 2020b; Zhang et al., 2020). LH and FSH levels fluctuate during the day and are changing continuously by various factors such as stress, exercise, and sleep patterns. These fluctuations may not accurately reflect spermatogenesis in the testes (McCosh et al., 2019). The response to hormonal signals may differ individually, so there can be variability in LH and FSH levels among men with similar reproductive conditions. This variability makes it challenging to consider hormones as predictors of micro-TESE outcomes.

We demonstrated that other parameters, in contrast to previous studies, such as age (Enatsu et al., 2015; Zarezadeh et al., 2021) and testicle size (T. J. Kim & Koo, 2023), do not have any statistically significant prognostic values. Because seminiferous tubules are found in the testis and are abundant, whether small or large in size, and only a few tubules are removed from the testicle during the procedure, the testicular volume appears to have no significant effect on outcomes, neither positively nor negatively.

In our study, the limited sample size is a significant limitation. The results, while indicative, should be interpreted with caution due to the possibility of sample bias. This limitation is consistent with the diversity observed in the literature, which demonstrates significant effect heterogeneity. Larger, multicenter investigations are needed to validate these findings and reach more definitive conclusions.

It would also be beneficial to consider more factors that may affect the spermatogenesis like any prior history of scrotal hernia, torsion, or testicular trauma as these conditions may have an impact on micro-TESE outcomes.

In this study, smoking was categorized based on self-reported frequency. Participants were asked about their smoking habits, including whether they smoked at least one pack of cigarettes daily, occasionally, or rarely. However, the study did not quantify smoking intensity (e.g., number of cigarettes per day or pack-years), which may have an important impact on fertility outcomes. Future research should explore the dose–response relationship between smoking and sperm retrieval success by collecting detailed smoking histories, including the duration and intensity of smoking exposure.

Conclusion

This study found that higher free testosterone levels and fewer previous micro-TESE efforts are significant predictors of effective sperm retrieval in males with NOA. However, the limited sample size restricts research findings' robustness and generalizability, emphasizing the need for more study with larger cohorts. Clinicians can improve outcome predictions by assessing free testosterone levels prior to surgery and thoroughly reviewing patients' histories of past micro-TESE attempts. The minimal relationship observed with testicular volume warrants additional investigation.

Moreover, the significant influence of testosterone levels and smoking on sperm extraction success highlights crucial public health considerations. Men undergoing fertility treatment should be informed of the impact lifestyle choices can have on reproductive outcomes. Strategies to maintain healthy testosterone levels include lifestyle changes, such as regular exercise, a balanced diet, stress management, and maintaining a healthy weight, or using medications and supplements. Smoking cessation should also be strongly recommended, as smoking has well-established detrimental effects on sperm quality and fertility. Physicians should incorporate smoking cessation support and hormone-level management into fertility care plans.

Future studies should investigate additional hormonal markers and stratify individuals based on the underlying cause of NOA. Larger, multicentric searches are needed to confirm these findings and increase the prediction accuracy for NOA patients undergoing micro-TESE.

Declaration of Conflicting Interests

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Ethical Considerations

Institutional review board approval was obtained (no. IRB/2023/515). No identifying information was used in the collected data.

Consent to Participate

All patients provided informed consent and voluntarily agreed to participate in this research study.

Consent for Publication

Consent for publication was obtained from all participants included in this study.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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