

REVIEW ARTICLE

Predictors of sperm retrieval with micro TESE, A narrative review article

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Abstract: **Introduction:** Regarding the financial and psychological effects of micro-TESE on patients with NOA, it is important to determine the parameters by which the sperm retrieval with micro-TESE can be predicted. **Methods:** The key words used for conducting a search in the PubMed database included nonobstructive azoospermia and TESE. The abstracts of the articles were reviewed, and the articles which reported the parameters' influence on sperm retrieval with micro TESE were included. All non-English papers, case reports, and case series, were excluded from the review. Eventually, 25 articles were selected to be included. **Results:** It is recommended that diagnostic test is biopsy should be performed at the time of micro-TESE. The histopathology of testis, testicular volume, hormone profile, aging, and genetic factors, are parameters that might influence the results of sperm retrieval with micro-TESE. Heterogeneous histopathological pattern and report of hypospermatogenesis on pathology, lower serum FSH level, normal testis volume, and varicocele, increase the chance of sperm retrieval with micro-TESE. In terms of sperm retrieval, some genetic factors such as AZFa, AZFb, and chromosome Y micro deletion provide poor prognosis. **Conclusion:** Testis histopathology is the most important factor which predicts sperm retrieval with micro-TESE. Other factors that influence the sperm retrieval rate are testis volume, genetic factor, serum FSH level, and history of varicocele.

Keywords: micro-TESE; nonobstructive azoospermia; sperm retrieval rate; testicular sperm extraction

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1. Introduction

Infertility is defined as inability to conceive after a year of unprotected, regular sexual intercourse. According to this definition, 15% of all couples suffer from infertility and need diagnostic workup. Azoospermia is defined as the absence of spermatozoa in the ejaculate and is diagnosed when no sperm can be found in two consecutive semen analysis. Azoospermia can be subdivided into Obstructive and Nonobstructive Azoospermia (NOA). It is important to distinguish whether the lack of sperm in the ejaculate is from an obstructive or non-obstructive process. Obstructive azoospermia is caused by obstructive conditions which prevent any delivery of sperm during ejaculation, like vasectomy. While nonobstructive azoospermia is caused by failure

of spermatogenesis [2, 3].

NOA affects approximately 1% of all men, and up to 15% of infertile men suffered from NOA [2]. For achieving pregnancy, these patients benefit from Intracytoplasmic Sperm Injection (ICSI) with micro-TESE procedure [4]. Various sperm retrieval techniques have developed over the years. These include percutaneous testicular biopsies with sperm retrieval rates (SRR) of 20% and micro-TESE with sperm retrieval rates of 63% [5]. An unsuccessful micro-TESE and ICSI procedure could cause financial and emotional difficulties [6]. Therefore, it is important to determine predictive factors for successful sperm retrieval. Multiple parameters such as serum hormones (Inhibin B, Testosterone, and Follicle-stimulating hormone), testicular size, and testicular histopathology, have been proposed as predictive factors for micro-TESE successful sperm retrieval. However, none of them have been proved to be reliable [7, 8]. In this article, we review papers which report on such parameters' influence on sperm retrieval with micro TESE. These papers were published in PubMed, from 2010 to 2020.

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2. Methods

The key words used for conducting a search in the PubMed database included nonobstructive azoospermia, micro-TESE and sperm retrieval. 52 articles were found. The abstracts of the articles were reviewed, and the articles which reported the parameters' influence on sperm retrieval with micro TESE were included. All non-English papers, case reports, and case series, were excluded from the review. Eventually, 25 articles were selected to be included. This was done in order to ensure that all articles about "micro-TESE sperm retrieval predictor factors" were enrolled. References from the included studies were manually retrieved to identify additional studies of interest.

3. Results

Several articles were published about factors which have an impact on the probability of sperm retrieval rate with micro-TESE in patients with non-obstructive azoospermia. Testicular biopsy results, testicular size, hormone profile (especially FSH), age, genetic factor, and other factors such as varicocele, are predictors of sperm retrieval rate in patients with non-obstructive azoospermia (table 2). In recent years, EAU guidelines have been recommending concurrent testicular biopsy and micro-TESE.

4. Discussion

4.1. Parameters influence sperm retrieval with micro TESE

4.1.1. Testicular biopsy results

Patients with azoospermia are at risk of developing testicular cancer [9]. Therefore, the purpose of testicular biopsy in these patients are not only to obtain sperm for assisted reproductive techniques particularly in vitro fertilization (IVF), but also to rule out intratubular germ cell neoplasia, if existed [10, 11]. Thereby, testicular biopsy, as a part of micro-TESE procedure, can be used for diagnostic purposes. Testicular histopathology is classified under the four categories of maturation arrest (MA) (early or late), Sertoli cell only (SCO), hypospermatogenesis, and tubular hyalinization. If only a singular histopathological biopsy pattern is obtained from the sample, it is considered a homogeneous pattern. Whereas, biopsies consisting of more than one pattern such as MA and SCO is considered heterogeneous [12].

Based on histopathological pattern, SRRs are varied. Hypospermatogenesis group reached an SRR of 94%, whereas the MA and SCOS groups had rates of 43.5% and 21.6%, respectively [8, 13]. Yang Yu, MSc et al. demonstrated sperm retrieval rates on testicular biopsy to be significantly higher in patients with heterogeneous pattern compared to patients with homogenous pattern (65% vs 15% with P-value <.001)

[12]. In patients with homogenous pattern, contralateral testis SRR are significantly lower than those with heterogeneous pattern (25% vs 3%; P-value =.036) [12]. Bernie et al. [14] showed that patients with late and focal MA had higher probability of sperm retrieval compared to those with diffuse and early MA (78% vs 40%; 57% vs 35%). This is consistent with the idea that the probability of sperm retrieval in patients with heterogeneous pattern is higher. Intraoperatively more heterogeneous tubules were consistent with the more likelihood of sperm retrieval procedure. Homogeneous tubules are considered to be an effective guide in the process of intraoperative decision making. For reducing complications, a limited (superficial) contralateral micro-TESE should be considered when a homogeneous pattern of tubules is demonstrated under optical magnification [12]. Yalcin Kizilkan et al. reported that 12% of patients who had sperm on previous biopsy had no sperm in the micro-TESE. Similarly, in 32% of patients who did not have sperm with previous biopsy, sperm retrieval was successful with micro-TESE (13,55). These findings provide a challenging outlook regarding the necessity of biopsy prior to micro-TESE. EAU Guidelines recommend concurrent testicular biopsy and micro-TESE in determining the cause of NOA and the histopathology [10].

4.1.2. Testicular size

With the exception of men with histology of late or early maturation arrest, [15, 16] patients with NOA usually have small testis [17]. Previously it has been proposed that men with significantly lower testicular volumes had impaired testicular function, and thus a decreased chance of SRR with micro-TESE. The nomogram was published to predict SRR with micro-TESE whereby the chances of sperm retrieval in a 40-year-old man, with Klein filter syndrome (KS) and 2 cc testis volume, is about 70-80%. Whereas the SRR decreased to less than 50% if he did not have KS [18]. Regarding the chance of sperm retrieval, several studies reported different cut-off values for testis volume (table 1). According to the 2019 guidelines of European Association of Urology, no cut-off value for testicular volume has been specified to ensure successful sperm retrieval [19]. Currently, it is accepted that regardless of their testicular volume, in men with azoospermia, micro-TESE can be proposed as a therapeutic option [11].

4.1.3. Hormone profile (FSH, LH, TESTOSTERONE, INHIBIN B (IHHB))

Given the emotional and financial burdens of micro-TESE, using noninvasive parameters to predict sperm retrieval with micro-TESE would be beneficial. Serum FSH levels are correlated with the total spermatogonia in seminiferous tubules. However, it cannot predict small focus of spermatogenesis within testis [25]. Jahromi BN reported that FSH plasma levels above the cut-off value of 14.6 mIU/ml are a predicting

factor for unsuccessful micro-TESE in NOA patients [26]. Ramasamy et al. demonstrated that in men with NOA, there is no straightforward relationship between serum FSH levels and the presence of spermatogenesis. They also showed that serum FSH level is a poor predictive factor in determining SRR with micro-TESE [25]. Multivariate logistic regression analysis revealed that there is no significant association between FSH, LH, testosterone level, and SRR [25]. Some studies reported that serum inhibin B level is a better predicting factor than FSH, for successful micro-TESE in patients with elevated FSH, and does not provide further information compared with FSH in patients with normal FSH. Therefore, its use is limited to patients with elevated FSH [27]. Hormone level depends on age and the time of blood sampling. For instance, INHB concentrations were higher in samples collected in the morning than those collected in the afternoon [28, 29]. Due to its high cost, measuring inhibin B levels is only possible in a limited number of patients [24]. According to the 2019 Guidelines of European Association of Urology, there is no association between serum FSH levels and SRR [8].

Intra Testicular Testosterone (ITT) serum is necessary for normal spermatogenesis [30]. However, some studies [31] found no association between serum testosterone levels of infertile men and the semen parameters. In saying that, some studies reported the success rates of micro-TESE to be lower in patients with preoperative testosterone levels less than 10 nmol/L compared to those whose testosterone levels are above this threshold ($P = 0.0068$) [32]. As suggested by various studies, through optimizing serum testosterone levels in NOA men with hypogonadism, improvement in micro-TESE results can be expected [24, 33]. Shiraishi et al. [33] found that in men with NOA, who experienced an unsuccessful micro-TESE sperm retrieval, human chorionic gonadotropin can have beneficial effects. Histological data suggested that men with late MA or hypospermatogenesis are likely to respond to hormonal treatment. Furthermore, some evidence showed that with hormonal therapy, histologic pattern can change from MA to hypospermatogenesis in men with positive response to hormonal therapy [32]. Therefore, hormonal assessment prior to micro-TESE is not an effective predictor of successful sperm retrieval.

4.1.4. Age

Nowadays, both men and women have weighed the age factor in terms of conceiving. There is no clear cut-off age for deterioration of male fertility [34-36], and the outcome of sperm retrieval in advanced age has not been outlined [37]. However, advanced paternal age may have adverse effects on sperm retrieval outcomes in KS patients [38]. Some studies reported that the probability of acquiring azoospermia (secondary azoospermia) in men older than 50 years is higher than those younger. Whereas congenital NOA is more prob-

able in younger men. The higher amounts of hypospermatogenesis in a diagnostic biopsy of men older than 50 years supported this theory. Sperms were successfully retrieved for most men who were older than 50 years [39].

4.1.5. Genetic factor

The detection of chromosome abnormalities might help the prediction of sperm retrieval in patients with NOA. Fifteen percent of patients with azoospermia have an underlying genetic disorder [40]. The most common of which are Klinefelter syndrome (KS) and Y chromosome micro-deletion. According to the 2019 guidelines of European Association of Urology, sperm can be retrieved in 30% of patients with KS [10]. Sabbaghian et al. [41], which analysed the highest number of cases so far, reported that sperm could be retrieved with micro-TESE in 28.4% of 134 patients with KS.

Given the fact that the widespread apoptosis of spermatogonia happens at the onset of puberty in patients with KS, it seems that the chance of finding spermatogonia on biopsy is most likely at the age of 10 (near 100%), but declines to around 40% when patients transition to early adulthood [42]. Sperm retrieval procedures before puberty cannot guarantee fertility in adulthood [43] and may even reduce the likelihood of fertility by eliminating immature germ cells which may develop into spermatozoa later in life. Others offer testis biopsy before puberty only if other surgeries such as orchiopexy or tonsillectomy were scheduled at that time [42].

Some studies showed that an undescended testicle in Klinefelter patients, increases the chance of germ cell loss. Therefore, it is logical to consider testicular biopsy at the time of orchiopexy in KS male patients [44]. Ethical complications should be taken into account when testicular biopsy is scheduled before puberty [45] as long-term effects of early testis biopsy are unknown. Therefore, testis biopsy in childhood should be considered as an experimental procedure.

The conclusive evidence proposed to perform micro-TESE to obtain sperm in KS patients between the age of 15 and 25 [46]. The second most common chromosomal abnormality seen in azoospermia patients is Y chromosome micro-deletion hovering around 10%-15%. There are 3 types of Y chromosome microdeletion, including AZFa, AZFb, and AZFc, the most common of which is AZFc deletion [47]. In terms of sperm retrieval, patients who harbor an AZFb deletion have the worst prognosis, and should be informed about the consequences of micro-TESE. However, patients who have AZFc microdeletion presented a high sperm retrieval rate with micro-TESE (approximately 60%) [48, 49]. Sperm retrieval in patients with AZFa and AZFb microdeletions is unlikely. Thus, alternatives such as donor insemination or adoption should be contemplated [50].

Regarding other genetic factors, SPEN1 as a post-meiotic marker is the best marker to predict the probability of sperm retrieval in patients with azoospermia [51].



Table 1: Different cut off values for testis volume

Total (%)	Incontinence
Amer et al [20]	Even in patients with testicular volumes of less than 5 ml, spermatozoa could be retrieved.
Turunc et al [21]	In testicular volumes less than 5 ml, SRR found to be 20.8%, 40% in volumes between 6 and 15 ml and 58.2% in testicular volumes of higher than 16 ml.
Manconi et al [22]	"low chance" (approximately 30%) of SRR using micro-TESE in patients with FSH levels >12.4IU and testicular volumes < 8mL.
Bromage et al [7]	Lower SRR in patients with FSH levels >10IU and testes volume <4mL with percutaneous testicular sperm extraction or epididymal sperm aspiration(29%)
Ziaee et al [23]	For testicular volume, cutoff points were determined to be 9.5 mL
Yalcin Kizilkan et al[24]	Increase in the SRR of micro-TESE with higher testicular volumes with volumes above 11 ml considered to have significant successful results.

Table 2: Factors predicting sperm retrieval with Micro TESE(summary)

Factors predicting SRR With Micro TESE	Conclusion
Histopathology	1-The chance of SRR with Micro TESE is the highest in hypo spermatogenesis group and the lowest in the SCOS group. 2-Sperm retrieval rates on testicular biopsy to be significantly higher in patients with heterogeneous pattern compared with patients with homogenous pattern.
Testicular size	1-No cut-off value for testicular volume has been specified to ensure successful 2-Sperm retrieval however the chance of SRR in patients with normal testis size is higher than patients with small testicular size.
Hormone profile	1-Serum FSH level is a poor predictive factor in determining SRR with micro-TESE 2-The use of inhibin B is limited to patients with elevated FSH but its cost limited its use in the practice. 3-Optimizing serum testosterone levels in patients with NOA and hypogonadism is encouraged before Micro TESE to improve results
Age	With exception of patients with KS, advanced age is not associated with the results of Micro TESE.
Genetic factor	1-The chance of sperm retrieval declined with age in patients with KS but Ethical issue should be considered when testicular biopsy is scheduled before puberty 2-Sperm retrieval in patients with AZFa and AZFb microdeletions is unlikely thus alternatives such as donor insemination or adoption should be contemplated but the patients who have AZFc microdeletion presented a high sperm retrieval rate with micro TESE
Other factors	Performing varicocelectomy had a useful effect on sperm quality of patients with NOA and can increase the SRR with micro-TESE.

4.1.6. Other factors

Several factors such as varicocele, smoking status, history of hernioraphy, history of orchidopexy, and duration of infertility, might have adverse effects on sperm retrieval rate [24]. Amongst these factors, only varicocele had considerable effects on sperm retrieval rate. Performing varicocelectomy proved effective on sperm quality of patients with NOA. It can increase the SRR with micro-TESE and improve the results of IVF-ICSI such as live birth rate [52, 53]. Age at orchidopexy, before or after 10 years of age, was not a predictive factor for successful TESE [54]. Patients with a history of cryptorchidism had better TESE sperm retrieval rates ($p < 0.05$), although no significant differences were observed [55].

5. Conclusion

Testis histopathology is the most important factor which predicts sperm retrieval with micro-TESE. Other factors that influence on sperm retrieval rate are testis volume, genetic factors, serum FSH level, and history of varicocelectomy (table 2). Aging and history of orchidopexy do not influence the chance of sperm retrieval with micro-TESE.

6. Appendix

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6.2. Author contribution

All the authors have the same contribution.

6.3. Funding/Support

None.

6.4. Conflict of interest

No conflict of interest.

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