# Evaluation of Microdissection Testicular Sperm Extraction Results in Patients with Non-Obstructive Azoospermia: Independent Predictive Factors and Best Cutoff Values for Sperm Retrieval

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**Purpose:** Testicular sperm extraction (TESE) for intracytoplasmic sperm injection (ICSI) was first introduced for the treatment of non-obstructive azoospermia. This study was conducted to detect predictive factors affecting the success of microTESE.

**Materials and Methods:** We retrospectively evaluated the results of 191 cases who underwent microTESE. For each patient, the testicular volume, endocrine profile [follicle stimulating hormone (FSH), luteinizing hormone (LH), free testosterone (FT), total testosterone (TT)], serum inhibin B level, karyotype analysis, and Y chromosome microdeletions were recorded, and all data were analyzed to detect any predictors. The receiver operating characteristic curve, two-sample *t*-test and regression analysis were used for the statistical analysis.

**Results:** The mean age of the patients was 34.4 ± 5.6 years. Sperm retrieval was successful in 104 (54.5%) patients, and there was no sperm in 87 (45.5%). Seven factors including, testicular size, Johnson score, Y chromosome microdeletion, and serum FSH, LH, FT and TT levels were different between the successful and unsuccessful groups. Six patients had Klinefelter syndrome, and ten patients (5.2%) had a Y chromosome microdeletion (5 AZF-c, 1 AZF-b, 2 AZF-bc, 1 AZF-abc, and 1 AZF-ac). The Johnson score, TT level, family history and Y chromosome microdeletions were determined to be independent predictive factors for sperm found. According to the testicular histology, the sperm-found ratios were 36%, 48.6%, and 95.5% in the sertoli cell only syndrome, maturation arrest, and hypospermatogenesis groups, respectively.

**Conclusion:** According to our results, the Johnson score, TT level, family history-related infertility, and Y chromosome microdeletions were determined to be independent predictive factors for sperm found.

**Keywords:** azoospermia; surgery; microdissection; methods; sperm retrieval; testis; male.

## INTRODUCTION

Azoospermia, which is the complete absence of sperm in the ejaculate, accounts for 10-15% of male infertility cases.[1] Known genetic factors are responsible for approximately 1/3 of cases of azoospermia. Nonetheless, at least 40% of cases are currently categorized as idiopathic and may be linked to unknown genetic abnormalities.[1] Azoospermia is classified as obstructive azoospermia (OA) or non-obstructive azoospermia (NOA), each having very different etiologies and treatments. Non-obstructive azoospermia constitutes 60% of all cases of azoospermia.[1]

Testicular sperm extraction (TESE) for intracytoplasmic sperm injection (ICSI) was first introduced for treatment of obstructive azoospermia in 1993.[2,3] Soon afterwards testicular sperm were retrieved successfully and used in ICSI in cases of NOA.[4-6] In the NOA cases, TESE combined with ICSI has been proven to be an acceptable line of treatment.[6] Independent of sperm retrieval via the microTESE, dependent predictive factors, genetic evaluations and the management of these situations, such as luteinizing hormone (LH), follicle stimulating hormone (FSH), inhibins, testicular volume, azoospermia factor (AZF) regions, and Klinefelter syndrome, were reported in a variety of studies.[7-10] In our study, we evaluated the results of microTESE in patients with NOA and determined the factors affecting the success of microTESE.

## MATERIALS AND METHODS

### *Study Population*

We retrospectively evaluated 191 cases who had under-

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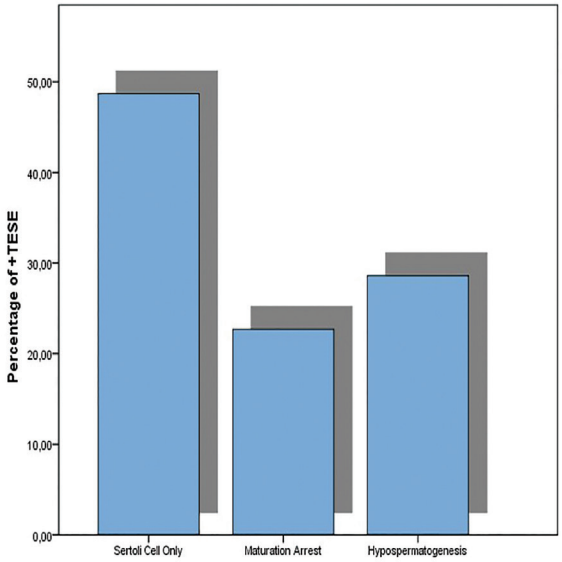


**Figure 1.** Percentage of positive Testicular sperm extraction (TESE) according to the histologic classification.

gone microTESE between December 2006 and 2009 in our institution. Informed consent was obtained to use these patient data for our study. In all patients diagnosed with NOA, data regarding testicular volume (measured using Prader-orchidometer ), FSH, LH, free testosterone (FT) and total testosterone (TT) levels, inhibin B, karyotype analysis and Y chromosome microdeletions were gathered from medical records. Patients with non-palpable vas deferens exclude from the study. In order to exclude OA our radiologist done transrectal ultrasonography in all patients with ejaculate volume lower than 2 mL. The patient's family history of infertility, include parents' and first degree relatives' spontaneous abortion and the inability to achieve pregnancy, was also recorded. Azoospermia in patients with normal-size testes and normal FSH suggested obstructive.

### *Seminal Study*

Semen samples were produced by masturbation after 3-6 days of sexual abstinence and collected into sterile containers. The azoospermia was confirmed by at least two seminal analyses (> 4 weeks apart) which were carried out as described in the World Health Organization (WHO) Manual (WHO 1999).[11]

### *Hormone Analyses*

Blood samples were taken from antecubital vein at morning in fasting situation. Hormones were measured using commercially available kits. Serum FSH concentrations were measured by an immunoenzymatic assay with two monoclonal antibodies (Immuno 1; Technicon, Bayer, Tarrytown, NY, USA), and the data were expressed in terms of International Reference Preparations (IRP)78/549. The sensitivity of the assay was 0.1 IU/L, and the inter-assay coefficient of variation was 2.7%. Plasma testosterone was analyzed by a radioimmunoassay (Diagnostic Products Ltd, Wales, UK), according to the manufacturer's instructions. Dimeric inhibin B was measured by a solid-phase sandwich enzyme-linked immunosorbent assay, which used two monoclonal antibodies (Serotec, Oxford, UK).

### *Fluorescent Polymerase Chain Reaction (FL-PCR)*

Four multiplex FL-PCR formats were developed, including a total of 28 different primer pairs to screen different loci dispersed on AZF a, b, and c, SRY, ZFX/ZFY and the Y distal heterochromatin region. PCR was performed on genomic DNA extracted from peripheral blood cells, and the products were visualized by agarose gel electrophoresis as previously described.[12]

### *Microdissection TESE*

The microTESE procedure was performed under a 20 to 40 × magnification operating microscope. An attempt was made to identify individual seminiferous tubules that were larger and more opaque than other tubules in the testicular parenchyma. Small samples (15–20 mg from each testis) were excised from the larger, more opaque tubules.

### *Sperm Retrieval*

Each sample was placed in a petri dish filled with 0.5 mL of human tubal fluid (HTF) medium, minced and shredded using sterile glass slides. Then, each sample was examined immediately by placing a small droplet

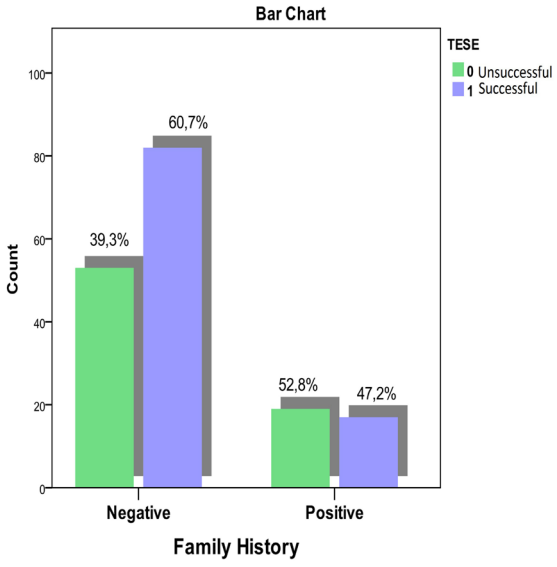


**Figure 2.** Family history as an independent predictive factor for sperm retrieval.
**Abbreviation:** TESE, testicular sperm extraction.

**Table 1.** Characteristics of study subjects

| Variables | Microdissections Testicular Sperm Extraction | | | *P* Value |
|---|---|---|---|---|
| | All patients | Unsuccessful group (n = 87) | Successful group (n = 104) | |
| Age (years) | 34.4 ± 5.6 | 33.8 ± 5.4 | 34.9 ± 5.8 | .203 |
| Duration of infertility (years) | 7.8 ± 5.1 | 7.56 ± 4.8 | 8.1 ± 5.3 | .512 |
| Testicular size (mL) | 9.74 ± 8.09 | 7.22 ± 6.1 | 11.7 ± 8.9 | .001* |
| Endocrine profile | | | | |
| FSH (mIU/mL) | 21.1 ± 15.1 | 24.9 ± 15.2 | 17.5 ± 14.1 | .001* |
| LH (mIU/mL) | 8.9 ± 6.5 | 11.0 ± 7.5 | 7.1 ± 4.9 | .001* |
| Free testosterone (pg/dL) | 13.1 ± 8.4 | 11.1 ± 7.2 | 14.7 ± 8.9 | .004* |
| Total testosterone(ng/mL) | 420.3 ± 265.1 | 367.5 ± 258.7 | 468.7 ± 263.7 | .023* |
| Prolactin (ng/dL) | 13.2 ± 18.1 | 11.3 ± 7.4 | 14.9 ± 24.1 | .617 |
| Inhibin-B (pg/dL) | 134.5 ± 144.7 | 114.4 ± 139.5 | 153.5 ± 148.1 | .078 |
| Histopathology (Johnsen score) | 4.0 ± 2.8 | 2.5 ± 1.5 | 5.4 ± 3.1 | .000* |
| Y-chromosome microdeletions, % | | | | |
| No | | 89.7 | 99.0 | |
| Yes | | 10.3 | 1.0 | .006* |
| Patient with varicocele, % | | | | |
| No | | 83.5 | 83.7 | |
| Yes | | 16.5 | 16.3 | .982 |
| Previous inguinal and scrotal surgery | | | | |
| No | | 52.9 | 54.8 | |
| Yes | | 47.1 | 45.2 | .672 |
| Family History | | | | |
| Negative | | 73.6 | 82.8 | |
| Positive | | 26.4 | 17.2 | .144 |

**Abbreviations:** LH, luteinizing hormone; FSH, follicle stimulating hormone.
* Statistically significant.

of the dispersed tissue suspension on a slide under a phase microscope using 200 × magnification for the presence of testicular sperm. A small sample was taken for histological diagnosis.

### *Histopathology*

Tissue sections were fixed in Bouin's solution, stained with hematoxylin andeosin, and examined by the same expert pathologist under the microscope. Testicular histology was classified as previously reported into hypospermato genesis, maturation arrest (MA) and sertoli cell only (SCO).[13] The testicular histology was scored on a scale of 1-10 according to the method of Johnson.[14]

### *Statistical Analysis*

The Statistical Package for the Social Science (SPSS Inc, Chicago, Illinois, USA) version 18.0 was used for the statistical analysis. Power analysis was performed. The clinical factors were analyzed with the independent sample *t*-test, Mann Whitney *U*, chi-square, multivariate regression analysis, and Fisher's exact tests. The receiver operating characteristic (ROC) curve analysis was used to determine the best cutoff values. A value of $P < .05$ was considered statistically significant.

## RESULTS

A total of 191 patients underwent microTESE. The sample size was adequate for a power size of 80% and alpha of 0.05 according to the power analysis for regression (n = 191). The mean age of the patients was 34.4 ± 5.6 years; the mean age was 33.8 ± 5.4 years in the unsuccessful group and 34.9 ± 5.8 years in the successful group. The sperm retrieval was successful in 104 patients and unsuccessful (no sperm found) in 87 patients. The overall sperm retrieval rate was 54.5%.

**Table 2.** Best cutoff predicted probability with respect to sensitivity and specificity with receiver operating characteristic curve.

| Variables | AUC (95% CI) | Best Cutoff Value | Sensitivity % | Specificity % | *P* Value | PPV | NPV | LN+ | LN- |
|---|---|---|---|---|---|---|---|---|---|
| Testicular size (mL) | 0.658 | 10 | 77.8 | 49 | .002 | 0.62 | 0.38 | 1.65 | 1.60 |
| FSH (mIU/mL) | 0.656 | 15 | 75 | 51.2 | .001 | 0.60 | 0.40 | 1.52 | 1.47 |
| LH (mIU/mL) | 0.666 | 7.5 | 63.1 | 63.9 | .001 | 0.50 | 0.49 | 1.03 | 1.00 |
| Free testosterone (pg/dL) | 0.652 | 11 | 66.7 | 63.4 | .004 | 0.52 | 0.48 | 1.08 | 1.05 |
| Total testosterone (ng/mL) | 0.648 | 400 | 52.2 | 60 | .023 | 0.47 | 0.53 | 0.89 | 0.86 |
| Histopathology (Johnsen score) | 0.790 | 2 | 71.2 | 74.7 | .000 | 0.49 | 0.50 | 0.99 | 0.96 |

**Abbreviations:** LH, luteinizing hormone; FSH, follicle stimulating hormone; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratios; LR−, negative likelihood ratios; AUC, area under the curve; CI, confidence interval.

According to the histological evaluation, the frequency of SCO, MA and hypospermatogenesis was 48.7%, 22.7%, and 28.6%, respectively (**Figure 1**).

### *Comparison between the Successful and Unsuccessful Outcomes*

The values for the 13 clinical factors, which were analyzed using Student's *t*-test, Mann Whitney *U*, chi-squared and Fisher's exact tests are shown for the successful and unsuccessful microTESE groups in **Table 1**. Seven factors, which were testicular size (volume), Johnson score, Y chromosome microdeletion, and serum FSH, LH, FT and TT levels, were significantly different between the groups according. However, the varicocele, previously inguinal and scrotal surgery, and family history ratios were similar between the groups (**Table 1**). The karyotype analysis of the patients (191) revealed that 6 patients had Klinefelter syndrome (47-XXY). Remaining patients had normal karyotypes (46-XY). Sperm was found in one patient (20%) with Klinefelter syndrome.

The Y chromosome microdeletion screen of the 191 patients revealed that 10 (5.2%) patients had a Y chromosome microdeletion (AZF deletion). One of these patients was in the successful group, and the other nine were in the unsuccessful group. The distribution of the AZF deletion regions was 5 AZF-c, 1 AZF-b, 2 AZF-bc, 1 AZF-abc, and 1 AZF-ac.

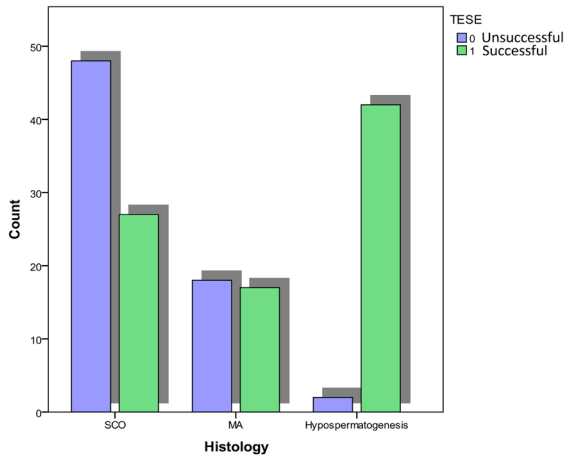


**Figure 3.** The sperm-found ratios according to the testicular histology.
**Abbreviations:** TESE, testicular sperm extraction; SCO, sertoli cell only syndrome; SA, spermatogenesis arrest.

### *Best Cutoff Predicted Probability with Respect to Sensitivity and Specificity with ROC Curve*

Of the seven clinical factors, the Y chromosome microdeletion results were excluded, and the ROC analysis was performed for 6 clinical factors. These analyses are shown in **Table 2**. The best cutoff value of the serum FSH concentration for discriminating between successful and unsuccessful TESE was 15 mIU/mL (sensitivity 75%, specificity 51.2%, $P = .001$), with an area under the curve (AUC) of 0.656. The best cutoff value of the serum LH concentration for discriminating between successful and unsuccessful TESE was 7.5 mIU/mL (sensitivity 63.1%, specificity 63.9%, $P = .001$), with an AUC of 0.666. The best cutoff value of the serum FT concentration for discriminating between successful and unsuccessful TESE was 11 pg/dL (sensitivity 66.7%, specificity 63.4%, $P = .004$), with an AUC of 0.355. The best cutoff value of the serum TT concentration for discriminating between successful and unsuccessful TESE was 400 ng/mL (sensitivity 52.2%, specificity 60%, $P = .023$), with an AUC of 0.648. The best cutoff value of the testicular size for discriminating between successful and unsuccessful TESE was 10 mL (sensitivity 77.8%, specificity 49%, $P = .002$), with an AUC of 0.658. The best cutoff value of the Johnson score (range 1 to 10) for discriminating between successful and unsuccessful TESE was 2 (sensitivity 71.2%, specificity 74.7%, $P = .001$), with an AUC of 0.79.

### *Multivariate Analysis*

Independent predictive factors were detected by multivariate and regression analyses for the presence of

sperm in the TESE. Two different models were used for the analysis. The first model included all parameters (FSH, LH, testicular volume, inhibin B, family history, TT, FT, AZF deletions, prolactin, varicocele presence, and patient age) for the unknown testicular pathologic evaluation. In addition to the above mentioned parameters, model two included the testicular Johnson score and histology of the patients who previously underwent testicular biopsy.

According to model one, FSH, TT, family history, and Y chromosome microdeletions were independent predictive parameters for sperm retrieval (**Table 3**). Furthermore, in model two for patients who previously underwent testicular biopsy, the Johnson score, TT, family history, and Y chromosome microdeletions (except for the AZF c microdeletion) were determined to be independent predictive factors for sperm found (**Table 3**). A positive family history was also found to be an independent predictive factor for sperm retrieval (**Figure 2)**. According to the testicular histology, the sperm-found ratios were 36%, 48.6%, and 95.5% in the SCO, MA and hypospermatogenesis groups, respectively (**Figure 3**).

## DISCUSSION

The first treatment modality of cases with NOA is TESE combined with ICSI. The success rates were between 24% and 81% in patients with NOA.[13] Unsuccessful microTESE can cause psychological, financial and physical distress in couples. Therefore, determining predictive factors for successful sperm retrieval has become important. Similar studies were conducted previously to determine predictive factors in patients with NOA, and different formulas were developed by various authors.[10] In many studies, the testicular size, endocrine profile, testicular histology, patient history, and genetic evaluation were considered to be predictive factors.[8,10] In terms of sperm retrieval techniques, the success of microTESE compared with conventional TESE has been reported in the literature.[4,15]

There was a negative correlation between elevated FSH and LH levels and spermatogenesis. Increasing FSH and LH levels were found to have poor predictive values for successful TESE.[8] In our study, we found statistical differences in the serum FSH and LH levels between the successful and unsuccessful groups (**Table 1**). The best cutoff points for FSH and LH were calculated to be 15 mUl/mL (sensitivity 75%, specificity 51.2%, $P$ = .001) and 7.5 mUl/mL (sensitivity 63.1%, specificity 63.9%, $P$ = .001), respectively. In contrast, similar studies did not suggest these results.[9,16,17] Additionally, LH was not found to be predictive factor for TESE in each regression model. However, FSH was determined to be an independent predictive factor for sperm found in regression model one, which did not include testicular histology. In model two, FSH did not affect the success of TESE because the FSH was already reflected in the testicular histology and was highly correlated with the Johnson score. The total testosterone level was found to be an independent predictive factor for sperm found in published studies.[10] In the present study, we also found that total testosterone was an independent predictive factor for sperm retrieval in each regression model, and we detected significantly different levels between the groups in the chi-square test (**Table 1**).

Inhibin B is accepted as reflecting spermatogenesis. It is secreted primarily from Sertoli cells, and the serum inhibin B level reflects the function of the seminiferous tubules. It also has a negative feedback regulatory role between hypophysis and the gonads.[18] Published stud-

**Table 3.** Regression analysis models and independent predictive factors that reflect possibility of sperm retrieval.

| Models | Factor | Coefficients | 95% CI | | *P* Value |
|---|---|---|---|---|---|
| Model 1 | Constant | .757 | .535 | .980 | .000 |
| R Square 0.42 | FSH | -.013 | -.020 | -.006 | .000 |
| | TT | .001 | .000 | .001 | .005 |
| | Positive family history | -.387 | -.624 | -.149 | .002 |
| | Y Chromosome microdeletion | -.440 | -.795 | -.085 | .016 |
| Model 2 | Constant | .360 | .051 | .668 | .023 |
| R square 0.68 | Johnson | .220 | .109 | .332 | .000 |
| | Positive family history | -.355 | -.562 | -.149 | .001 |
| | Y Chromosome microdeletion | -.472 | -.813 | -.131 | .008 |
| | TT | .001 | .000 | .001 | .024 |

**Abbreviations:** LH, luteinizing hormone; FSH, follicle stimulating hormone;CI, confidence interval; TT, total testosterone.

ies indicate that serum inhibin B combined with FSH is a more sensitive marker than either serum FSH or inhibin B alone for disturbed spermatogenesis in men.[8,16,19] Some studies confirm that when inhibin B is used alone or together with FSH, it cannot predict sperm retrieval from testicular tissue samples.[10] However, inhibin B values cannot predict the type of spermatogenic damage. In addition, many studies have shown that in cases with focal SCO, the inhibin B and FSH rates are normal.[16,19,20] Meachem and colleagues found that using inhibin B alone or in combination with FSH cannot be helpful to decide whether to perform TESE on a patient.[21] In a study conducted by Ballesca and colleagues, inhibin B could discriminate between successful and unsuccessful TESE.[22] The difference between successful and unsuccessful TESE in cases with NOA compared with the control group, as determined by ROC analysis, was 40 pg/mL inhibin B, with a sensitivity of 90% and specificity of 100%. Pierik and colleagues also reported that serum inhibin B levels were significantly correlated with testicular biopsy scores and argued that inhibin B was the best available spermatogenetic serum marker.[23] Bellesca and colleagues reported that it is necessary to evaluate inhibin B in addition to the FSH level and karyotype analysis before performing TESE on a man with NOA.[22] In contrast, many studies showed that inhibin B did not have any role in predicting the presence of sperm before the TESE.[16,24]

In our study, the mean inhibin B levels were 153.5 pg/mL and 114.4 pg/mL in the successful and unsuccessful groups, respectively. There was no significant difference between the successful and unsuccessful groups in terms of the mean serum inhibin B level (**Table 1**). In the multivariate regression analysis, inhibin B was not found to be an independent predictive factor and thus cannot reflect sperm retrieval in either model. In our study, the inhibin B levels were different between each histopathological group (Kruskal-Wallis test $P = .01$). The inhibin B level appeared to reflect testicular histopathology, but the distribution of the inhibin B level was non-parametric, meaning a wide variety and irregular distribution. For this reason, the inhibin B level may not be an independent predictive factor in the regression model. The published data support this interaction between inhibin B and testicular histology. Erkardstein and colleagues detected different inhibin B levels according to the testicular histology.[16] Therefore, the sperm found rate was approximately 68% explained by model two.

The Y chromosome microdeletion is a reason for the spermatogenesis failure that causes male infertility. After Klinefelter syndrome, Y chromosome microdeletions are the second most common genetic reason for male infertility.[25] Over the last ten years, many studies that defined microdeletions in infertile patients have been performed, and the molecular diagnosis of deletions has been a routine diagnostic test for male infertility. The incidence of Y chromosome microdeletions ranges widely, between 1% and 55%. This rate has been reported as 15-20% in males with NOA. The highest deletion rate is reported for the azoospermic patient group.[26,27] In our study on patients with NOA (n = 191), ten patients (5.2%) had a Y chromosome microdeletion, with only one (1%) of these individuals in the successful TESE group and nine (10.7%) in the unsuccessful group. Between these two groups, there was a significant difference ($P = .006$) with respect to genetic damage. In one patient with a deletion (AZF-c deletion), sperm was found. In addition to the existence of a deletion, the location of the deletion is also important because sperm can be retrieved in those patients with an AZF-c deletion.[28] Recently, TESE has not been advised for patients with AZF-a or AZF-b deletions. Y chromosome microdeletion analysis has been suggested as a routine test before TESE for azoospermic or severe oligozoospermic patients.[28,29] Additionally, a positive family history (e.g., aborted, dead, malformed, mentally retardation children) was detected in 28% of the infertile population relatives.[30] Positive family history means: if the patients relatives have infertility history or death, aborted, and mentally retarded child. That means abnormal reproduction. However, a positive family history was not evaluated as an independent predictive factor for the success of microTESE. In this study, we determined that a positive history was an independent predictive factor for sperm retrieval (**Table 3**). Sperm was most likely present in patients without a positive family history (**Figure 2**). The chi-squared test for family history was insignificant, but in multivariate analysis, a negative family history was an independent high positive predictive factor for the sperm found group. This study is the first to demonstrate that family history is an independent predictive factor for the success of microTESE using multivariate analysis. Underlying genetic abnormalities other than Y chromosome microdeletions should affect spermatogenesis because the multivariate analysis showed that both AZF microdeletions and family history were found to be predictive factors for sperm retrieval. In this study, we concluded that genetic abnormalities significantly affect the TESE results except for AZF microdeletions or karyotype.

There was a relationship between testicular volume and

spermatogenesis. However, this relationship between testicular volume and pathology was not correlated under some conditions because topographic changes can occur.[10] Previous studies demonstrated that testicular volume was not an independent predictive factor for sperm retrieval.[7,9] In the present study, we found that the testicular volume was higher in the successful group than in the unsuccessful group but that the testicular volume was not an independent predictive factor in the multivariate analysis. In a recent study, the best cutoff value for testicular volume was calculated to be 10 mL. Ziaee and colleagues reported that testicular volume was a predictive factor for sperm retrieval and that the best cutoff value was 9.5 mL.[8] Although there were testicular topographic differences, this observation did not alter our significant differences between testicular volume and sperm retrieval. Testicular histology was one of the most important predictive factors for sperm retrieval.[31] The published data demonstrated that the probability of finding mature spermatozoa during TESE was significantly affected by the testicular histology. The best sperm retrieval rates occurred in patients with hypospermatogenesis, and low rates were found in patients with SCO.[32] In our study, we demonstrated similar findings: histology and the Johnson score were determined to be predictive factor for sperm retrieval according to our multivariate analysis. But we don't recommend testicular biopsy before microTESE. Because bad testicular histology is not contraindication for micro TESE. Known testicular histology can help to predict outcomes and we can share our predictions to the family. And also based on testicular histology, redo microTESE can be recommended if first microTESE was negative.

## CONCLUSIONS

According to our results, FSH and TT levels, family history, and Y chromosome microdeletion are independent predictive factors for sperm retrieval. Furthermore, previous testicular biopsy, Johnson score, TT level, family history, Y chromosome microdeletions, and inhibin B are independent predictive factors for sperm found. For the first time, we demonstrated that family history is a novel independent predictive factor for microTESE.

## CONFLICT OF INTEREST

None declared.

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