



Do body mass index, hormone profile and testicular volume effect sperm retrieval rates of microsurgical sperm extraction in the patients with nonobstructive azoospermia?

Nonobstrüktif azoospermisi olan hastalarda vücut kitle indeksi, hormon profili ve testis hacmi, mikrocerrahi sperm ekstraksiyonunda sperm elde etme oranlarını etkiler mi?

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Cite this article as: Karamazak S, Kızılay F, Bahçeci T, Semerci B. Do body mass index, hormone profile and testicular volume effect sperm retrieval rates of microsurgical sperm extraction in the patients with nonobstructive azoospermia? Turk J Urol 2018; 44(3): 202-7.

ABSTRACT

Objective: We aimed to evaluate the effect of body mass index (BMI), follicle stimulating hormone (FSH), and luteinizing hormone (LH) levels and the mean testicular volume on sperm retrieval rates in microsurgical sperm extraction (microTESE) in the patients with nonobstructive azoospermia (NOA).

Material and methods: The data of 282 infertile patients with NOA were analysed retrospectively. The patients with normal karyotype and no Y microdeletions were included in the study. The patients were classified according to their BMI scores and the medical history, physical examination and hormonal parameters were evaluated. The overall data were processed statistically with chi-square and logistic regression analysis and the relation between preoperative findings and sperm retrieval rates was investigated.

Results: The sperm retrieval rate of 282 patients after microTESE was found as 41.1%. There was no statistically significant difference in sperm retrieval rates among the subgroups classified according to BMI. FSH and LH levels and the mean testicular volume and pathologic findings were significantly correlated with sperm retrieval rates.

Conclusion: Finally significant correlation was determined between sperm retrieval rates and FSH, and LH levels and testicular volumes but no statistically significant difference was found in sperm retrieval rates among BMI groups.

Keywords: Body mass index; follicle stimulating hormone; luteinizing hormone; nonobstructive azoospermia; sperm retrieval.

ÖZ

Amaç: Çalışmamızda, nonobstrüktif azoospermi (NOA) olan hastalarda mikrocerrahi sperm ekstraksiyonunda (MikroTESE) vücut kitle indeksi (VKİ), follikül stimüle edici hormon (FSH), luteinize edici hormon (LH) seviyeleri ve ortalama testis hacimlerinin sperm elde edilmesine etkisini değerlendirmek amaçlandı.

Gereç ve yöntemler: NOA olan 282 infertil hastanın verileri retrospektif olarak incelendi. Normal karyotipi olan ve Y mikrolelesyonu olmayan hastalar çalışmaya dahil edildi. Hastalar VKİ skorlarına göre sınıflandırıldı ve tıbbi öykü, fizik muayene ve hormonal parametreler değerlendirildi. Veriler, ki kare ve lojistik regresyon analizi ile istatistiksel olarak değerlendirildi ve ameliyat öncesi bulgular ile sperm elde edilmesi arasındaki ilişki araştırıldı.

Bulgular: MikroTESE sonrası 282 hastanın sperm elde etme oranı %41,1 bulundu. VKİ'ye göre sınıflandırılan alt gruplar arasında sperm elde edilmesi oranlarında istatistiksel olarak anlamlı fark yoktu. FSH ve LH düzeyleri, ortalama testis hacmi ve patolojik bulgular ile sperm elde edilmesi oranları arasında anlamlı korelasyon bulundu.

Sonuç: Sperm elde edilmesi oranları ile FSH, LH ve testis hacimleri arasında anlamlı bir ilişki saptanmış ancak VKİ grupları arasında sperm elde edilmesi oranlarında istatistiksel olarak anlamlı fark bulunamamıştır.

Anahtar Kelimeler: Vücut kitle indeksi; follikül stimüle edici hormon; luteinizan hormon; nonobstrüktif azoospermi; sperm elde edilmesi.

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*Our study was presented as
a "poster presentation" at
the 25th National Urological
Congress (6- October, 2016).*

*Çalışmamız, 25. Ulusal Üroloji
Kongresi'nde (6-9 Ekim
2016) "poster sunumu" olarak
sunulmuştur.*

Submitted:
22.03.2017

Accepted:
20.06.2017

Available Online Date:
21.12.2017

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Available online at
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Introduction

Azoospermia is defined as detection of no spermatozoa after semen samples were properly centrifuged and examined under microscope.^[1] Azoospermia is found in 1% of all men and this rate rises to 15% in infertile men.^[2] Distinction between obstructive and nonobstructive azoospermia should be made in all patients whose semen analysis revealed azoospermia.

Patients with azoospermia should be evaluated firstly with detailed history, physical examination (especially the status of the vas deferens and testicular size) and hormone profile to differentiate between obstructive and nonobstructive azoospermia, because treatment protocols of these two pathologies are different.^[3] Congenital bilateral absence of vas deferens (CBAVD) is a common cause of obstructive azoospermia. The diagnosis of CBAVD is based on physical examination where vas deferens are not palpated bilaterally on physical examination.^[4] Patients with small testicular volumes have primary or secondary testicular failure. Serum hormone tests including testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin should be done to make diagnosis.^[5] Severe germ cell failure may exist in patients with small testes and serum FSH concentrations two or three times of normal. Genetic tests should be performed in patients with azoospermia due to testicular failure to rule out chromosome abnormalities such as Klinefelter syndrome and Y chromosome microdeletions.^[6]

Testicular sperm extraction (TESE) or microscopic TESE (micro-TESE) operations are treatment options for patients seeking to have a child and considered as nonobstructive azoospermia (NOA).^[7] TESE is also an option for patients with obstructive disorders.^[8] However sperm can not be found by TESE in all patients. Many factors have been studied that can affect the availability of sperm. Predictive factors such as age, body mass index (BMI), disease history, toxic exposure, previous surgery, smoking, presence of varicocele, cryptorchidism history, testicular volume, genetic anomalies, levels of hormonal parameters that could effect the success of sperm retrieval have been investigated.^[9]

In recent studies, oligozoospermia risk increases 1.1 times and azoospermia risk increases 1.4 times in men with BMI of 25-29.9 kg/m² (overweight) compared to men with normal BMI. Oligozoospermia risk increases to 1.4 and azoospermia risk increases to 1.8 in men with BMI >30 kg/m².^[10] There is strong evidence that male obesity effects fertility adversely by changing hormone levels, molecular composition of sperm and sperm function.^[11] Although obesity has been shown to effect sperm morphology and motility, some studies have asserted that it has no effect on these parametres.^[12]

The aim of this study is to investigate whether male BMI has an impact on the success of sperm retrieval with TESE in patients who underwent micro-TESE due to NOA, as well as the relationship between history, physical examination, information obtained from hormonal and pathological findings, preoperative findings and the success of sperm retrieval.

Material and methods

Data of infertile patients who applied to our infertility clinic between January 2011 and December 2015 were retrospectively analyzed. All patients underwent TESE with the diagnosis of azoospermia. TESE operation was performed on 282 patients who met the study criteria for inclusion according to the information obtained from their files. That was the first TESE operation for all patients. Patients whose semen analysis was evaluated NOA according to World Health Organization (WHO), and chromosomal analysis revealed normal karyotype (46, XY) without Y-microdeletions were enrolled to the study and then underwent micro-TESE operation. The study was performed in compliance with The Declaration of Helsinki.

Information about the age, and occupation of the patient, his partner's age, duration of infertility, previous or present surgery (ies), trauma(s), disease(s), smoking and alcohol use, exposure to toxic substances, presence of infertile family members, and relatives, any hereditary disease and any previous operation or treatment due to infertility were retrieved from the infertility files. BMI value was calculated by dividing weight (kg) by square meter (kg/m²) of the height. Patients were classified based on BMI values as normal <25 kg/m², overweight (25-29.9 kg/m²) and obese (≥30 kg/m²), respectively. Information about volume and consistency of the testis, epididymis, vas deferens, varicocele and hydrocele based on physical examination were recorded. Testicular volumes were calculated according to Prader orchidometer. Ejaculate volumes were recorded from semen analysis. FSH, LH and testosterone levels were also recorded.

Sperm retrieval rate after micro-TESE and operated testicular side were recorded from the files. Pathology findings were also recorded. Testicular biopsies were reviewed by the pathologists and classified in 8 different categories as Sertoli cell-only syndrome (SCOS), maturation arrest, common/focal tubular sclerosis, germ cell hyperplasia, Leydig cell hyperplasia, focal spermatogenesis, hypospermatogenesis and normal spermatogenesis. An undersigned informed consent form was requested from patients prior to the operation.

Statistical analysis

All analyzes were performed using Statistical Package for the Social Sciences 15.0 (SPSS Inc.; Chicago, IL, USA) statistical software package. Frequencies and percentages for classifier vari-

Table 1. Measurements of some variables-1

BMI (kg/m ²)	n	%
<25	101	35.8
25-29.9	141	50.0
≥30	40	14.2
Orchiopexy		
Yes	18	6.4
No	264	93.6
Varicocele		
Yes	14	5.0
No	268	95.0
Smoking		
Yes	145	51.4
No	137	48.6
Post-TESE sperm		
Yes	116	41.1
No	166	58.9

BMI: body mass index; n: number; TESE: testicular sperm extraction

Table 2. Measurements of some variables -2

	Mean; SD	Median; Min-Max
Age (year)	33.5; 6.0	33.0; 22-60
FSH (mIU/mL)	20.8; 17.4	18.3; 0-118
LH (mIU/mL)	9.5; 6.9	8.0; 0.1-45
tTST (ng/mL)	3.7; 2.7	3.2; 0.1-36
Testicular volume (mL)	13.3; 5.1	15.0; 2-25
Ejaculate volume (mL)	2.8; 1.5	2.5; 0.1-13

SD: standard deviation; Min: minimum; Max: maximum; FSH: follicle stimulating hormone; LH: luteinizing hormone; tTST: total testosterone

ables, mean values for continuous variables, standard deviation, median and minimum-maximum values of descriptive statistics were calculated. The difference between the mean values of continuous variables between groups were tested by Independent Samples t Test. Chi-square test was used for the analysis of the relationship between two stacker variables. Receiver Operation Characteristic (ROC) analysis was performed for the sufficiency of the diagnostic tests to predict the presence of sperm prior to micro- TESE, and area under curve (AUC) was calculated. The cut-off values of the parameters which have predictive power were estimated by the Youden Index method. Finally, the effect of variables for explaining the presence of sperm before micro-TESE has been investigated by the multivariate analysis (logistic regression). The analysis was performed on the 95% confidence level. $p < 0.005$ was considered statistically significant.

Table 3. The characteristics of the groups according to BMI

	BMI (mean±SD)			p
	<25 kg/m ²	25-29.9 kg/m ²	≥30 kg/m ²	
Age	32.9±6.3	33.3±5.5	35.7±6.6	0.036
FSH (mIU/mL)	20.9±15.7	21.4±19.3	18.4±13.8	0.631
LH (mIU/mL)	9.3±6.1	9.9±7.8	8.1±5.9	0.349
tTST (ng/mL)	4.0±2.0	3.6±3.2	2.8±1.6	0.061
Testicular volume (mL)	13.3±5.4	13.3±4.9	13.6±5.4	0.922
Ejaculate volume (mL)	2.7±1.3	2.8±1.5	2.9±2.1	0.690
Infertility duration (month)	16.23	14.39	15.44	0.480
Orchiopexy (n)				
Yes	3	13	2	0.136
No	98	128	38	
Varicocele (n)				
Yes	7	6	1	0.474
No	94	135	39	
Smoking (n)				
Yes	50	69	26	0.178
No	51	72	14	

BMI: body mass index; SD: standard deviation; FSH: follicle stimulating hormone; LH: luteinizing hormone; tTST: total testosterone

Results

The data related to age, BMI, orchiopexy (if any), varicocele on inspection, smoking history, sperm availability after micro TESE, FSH, LH, and total testosterone levels, volume per ml of big testicles, ejaculation volumes of 282 patients are given in Tables 1 and 2. Patients with missing information about serum FSH (n=1), LH (n=5), and total testosterone (n=24) levels were left out of assessments while hormonal parameters were being analyzed.

One hundred and sixteen out of 282 (41.1%) patients had sperm after micro- TESE, and the remaining 164 patients (58.9%) had no sperm. Variable properties of individual groups according to BMI are given in Table 3. The characteristic features of the groups were similar and showed a homogenous distribution. Groups were homogenous in terms of age, hormone levels, testis and ejaculate volumes according to BMI. Duration of infertility was similar between groups. Sperm retrieval rates between groups were not statistically significantly different ($p=0.412$) (Table 4).

As an outcome of TESE operation, when the factors that affect sperm retrieval rates were examined, it was found that average

Table 4. Demographic characteristics of the groups with and without sperm retrieval using TESE

	Post-TESE Sperm		p
	Present	Absent	
Age (Mean±SD)	33.5±6.1	33.6±6	0.878
BMI (kg/m²)			
<25	41.6	58.4	0.412
25-29.9	38.3	61.7	
≥30	50	50	
FSH (mIU/mL) (Mean±SD)	15.4±13.1	24.6±18.9	<0.001
LH (mIU/mL) (Mean±SD)	7.3±4.3	11±8	<0.001
tTST (ng/mL) (Mean±SD)	4±3.6	3.4±1.7	0.089
Testicular Volume (mL) (Mean±SD)	14.5±5.2	12.5±4.9	0.002
Ejaculation Volume (mL) (Mean±SD)	2.6±1.8	2.9±1.3	0.253
Orchiopexy (%)			
Present	44.4	55.6	0.768
Absent	40.9	59.1	
Varicocele (%)			
Present	28.6	71.4	0.327
Absent	41.8	58.2	
Smoking (%)			
Present	36.6	63.4	0.108
Absent	46	54	

TESE: testicular sperm extraction; SD: standard deviation; BMI: body mass index;

FSH: follicle stimulating hormone; LH: luteinizing hormone; tTST: total testosterone

values of FSH ($p<0.001$) and LH ($p<0.001$) variables were significantly lower, while mean testicular volume was higher in the group where sperm retrieval was achieved after micro-TESE, ($p=0.002$). As for other variables, no difference could be found between patient groups with and without sperm (Table 4).

Cut-off values for FSH (8.65 mIU/mL), LH (12.95 mIU/mL) testicular volume (15.5 mL) were calculated. The outcomes of logistic regression analysis of three variables including sperm retrieval rates, FSH ($p=0.002$) and LH ($p=0.004$) were statistically significant, whereas testicular volume ($p=0.402$) was not statistically significant. It was found that probability of sperm retrieval after TESE was 2.7 times higher in cases with $FSH<8.65$ relative to cases with $FSH\geq 8.65$, while it is approximately 3 times higher in cases with $LH<12.95$.

The main factors that effected the result between the variables investigated were FSH and LH. In case of these variables are below cut- off value, higher testicular volumes also increase suc-

Table 5. Histopathological characteristics of sperms retrieved during TESE procedure in groups with and without sperm retrieval

	Characteristics of post-TESE Sperm				
	Present		Absent		p
	n	%	n	%	
Sertoli cell- only	29	14.2	175	85.8	204 <0.001
Maturation arrest	29	19.3	121	80.7	150
Germ cell aplasia + focal spermatogenesis	12	80	3	20	15
Hypospermatogenesis	76	77.6	22	22.4	98
Normal spermatogenesis	36	100	0	0	36
Tubular sclerosis + Leydig cell hyperplasia + germ cell hyperplasia	8	19.5	33	80.5	41
Atrophic testis	0	0	3	100	3

TESE: testicular sperm extraction; n: number

cess rates but this rise was not found to be statistically significant. In case of these variables above cut off value, achievement rates decrease independently of testicular volume.

Sperm detection rates of the patients were based only one pathology sample in 11 of 282 patients in whom micro-TESE was applied, because these patients had single testicles. Pathological evaluation could not be done due to inadequate biopsy specimens in 3 patients. Pathological evaluation was conducted with bilateral biopsies in 286 patients using micro- TESE. A total of 547 tissue samples were included in the study according to histopathological grading previously mentioned.

Sertoli cell-only (37.2%) pathology was detected mostly in histopathological examination. Maturation arrest (27.4%), hypospermatogenesis (17.9%), group with several rates of tubular sclerosis+Leydig cell hyperplasia+germ cell hyperplasia (7.4%), normal spermatogenesis (6.5%), germ cell aplasia+focal spermatogenesis (2.7%) and atrophic testicle (0.5%) pathologies were determined in respective percentages of patients. A statistically significant relationship was determined between sperm retrieval rates after micro- TESE and pathology results ($p<0.001$) (Table 5).

Discussion

There are many factors that affect sperm retrievals during micro- TESE operation and these factors may coexist in most of the patients. But these risk factors don't effect the availability of sperm at the same rate. Sperm detection rates can vary between 50% and 20%.^[13,14]

It was observed that pregnancy lasts longer in the couples with obese male partner.^[15] It was reported that there was a negative relationship between BMI, serum testosterone and sex hormone binding globulin in a meta-analysis.^[16] In our study, any statistical significant intergroup difference was not detected between average FSH, LH and total testosterone levels in terms of the groups classified according to BMI cut-off values. In Longitudinal Investigation of Fertility and the Environment (LIFE) study it was found that overweight and obese males had low ejaculate volumes, low sperm concentrations and high incidence of low total sperm counts.^[17] But in some studies correlations could not be detected between male obesity and sperm concentration.^[13] Mac Donald et al.^[18], could not find any significant correlation between BMI and the semen parameters measured with the exception of normal sperm morphology in their cohort of 511 patients. Wu et al.^[19] found that with increasing BMI, fertilization rates decreased proportionately; but embryonic cleavage rates and effective embryo rates were not significantly affected. In a recent study, increased BMI was found to affect semen parameters negatively even in fertile men.^[20]

In Ramasamy's^[21] study no significant difference was found between the groups in terms of mean FSH levels, testicular volume, presence of a varicocele, cryptorchidism, and Klinefelter's syndrome.^[21] Similarly, there was no statistically significant difference in terms of sperm detection rates among groups according to BMI in our study (41.6, 38.3, and 50%, respectively).

Seo et al.^[22] showed that sperm retrieval rates were not related to testicular size and FSH values, while it was correlated with histopathology in their study. Sperm detection rate was 52.8% in their study. It was found that pathology result was statistically significantly related to sperm detection rates in our study.

Ziaee et al.^[14] researched the relationship between the results of sperm detection with several parameters before TESE. It was detected that patients with testicular sperm had more testicular volume, higher serum inhibin level B and lower FSH levels. In our study, we found that the patients with higher sperm detection rates, had higher testicular volume and statistically significantly lower serum FSH levels.

In the study of Tsujimura et al.^[13], sperm detection rate was 42.9% and it was reported that serum FSH, total testosterone, and inhibin B levels were significant factors for sperm detection. We could not determine the relationship between total testosterone level and sperm detection rate in our study.

In the study of Bonarriba et al.^[23] recently, micro TESE was applied to total 74 patients with azoospermia. Sperm was detected in 36% of these patients. Significant relationship was determined between FSH, serum inhibin B and testicular histopathology and a weak relationship was detected with testicular volume which

was not proven statistically. In our study, cut-off value for FSH was 8.65 and it was found that sperm detection rates were 65.4% and 32%, below and above of this value, respectively.

In conclusion, many parameters which can be associated with sperm retrieval rates before TESE were investigated. Studies have shown that some physical symptoms and hormone levels may be linked with the success of sperm retrieval but this relationship is not clear in some studies. Increased BMI and obesity are controversial for effecting the spermatogenesis. For the moment, there is not a perfect parameter in order to predict sperm detection in TESE. Further studies are needed to reveal these values more clearly.

Ethics Committee Approval: Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – S.K., B.S.; Design – S.K., F.K., T.B., B.S.; Supervision – F.K., B.S.; Resources – S.K., F.K., T.B.; Materials – T.B.; Data Collection and/or Processing – F.K., T.B.; Analysis and/or Interpretation – S.K., F.K., T.B., B.S.; Literature Search – S.K., F.K.; Writing Manuscript – S.K., F.K.; Critical Review – F.K., B.S.; Other – S.K., F.K., T.B., B.S.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

Etik Komite Onayı: Yazarlar çalışmanın World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013) prensiplerine uygun olarak yapıldığını beyan etmişlerdir.

Hasta Onamı: Yazılı hasta onamı bu çalışmaya katılan hastalardan alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir – S.K., B.S.; Tasarım – S.K., F.K., T.B., B.S.; Denetleme – F.K., B.S.; Kaynaklar – S.K., F.K., T.B.; Malzemeler – T.B.; Veri Toplanması ve/veya İşlemesi – F.K., T.B.; Analiz ve/veya Yorum – S.K., F.K., T.B., B.S.; Literatür Taraması – S.K., F.K.; Yazıyı Yazan – S.K., F.K.; Eleştirel İnceleme – F.K., B.S.; Diğer – S.K., F.K., T.B., B.S.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

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