Experimental & Clinical Article

Reproductive Medicine; *Endocrinology and Infertility*

Can Testicular Size Be a Predictive Factor for Successful Sperm Retrieval in Patients with Non-Obstructive Azoospermia?

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ABSTRACT

OBJECTIVE: Non-obstructive azoospermia, defined as absence of spermatozoa in the ejaculate caused by impaired spermatogenesis, is the most severe cause of male infertility. It is typically presented as high serum follicle stimulating hormone levels and atrophic testis. The combination of intracytoplasmic sperm injection and Microdissection testicular sperm extraction allows these infertile men the opportunity to have their own children from their own testis. Our aim was to evaluate the outcomes of micro-Testicular sperm extraction in men with atrophic testis.

STUDY DESIGN: The medical records of 80 non-obstructive men with azoospermia who underwent micro-TESE were retrospectively evaluated. We assessed clinical parameters; age, duration of infertility, smoking, chromosomal karyotype, Y chromosome microdeletion, follicle stimulating hormone, luteinizing hormone, total testosterone and testicular volume in relation with Microdissection testicular sperm extraction results.

RESULTS: Testicular sperm retrieval rate was 53% in 80 patients. Testicular volume, serum follicle stimulating hormone and total testosterone concentrations showed correlation with the results of sperm retrieval. These three parameters were found to be significant risk factors with testicular sperm extraction negative patients (p<0.001). The odds ratios (95% CI) were 6.39 (1.25-26.58), 1.24 (1.11-1.36), 1.13 (0.99-1.21) respectively. Testicular volume was found to be a discriminative parameter in patients with negative sperm retrieval. The cut-off point was established as 6.75 mL for testicular volume with 88.1% sensitivity, 62.1% specificity.

CONCLUSION: Microdissection testicular sperm extraction is the most effective procedure for patients with non-obstructive azoospermia. Testicular volume, serum follicle stimulating hormone and testosterone levels can be predictive factors for sperm retrieval in men with non-obstructive azoospermia.

Keywords: Follicle stimulating hormone testosterone, Microdissection testicular sperm extraction, Non-obstructive azoospermia, Testicular volume

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Introduction

Azoospermia, defined as a complete absence of spermatozoa in the ejaculate, affects approximately 1% of the male population and 10-15% infertile males (1). It is mainly classified as obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). NOA affects approximately 60% of men with azoospermia, is associated with impaired spermatogenesis. Despite the absence of spermatozoa in the ejaculate, there are isolated focal areas of active spermatogenesis (2). Testicular sperm extraction (TESE) is often used for sperm retrieval from their own testis to have children. But one of the main disadvantages of this procedure is it may skip the active spermatogenetic areas (3). Microdissection testicular sperm extraction (micro-TESE) technique was first described by Schlegelin 1998. This is a less invasive technique and it gives a chance to visualize the active spermatogenetic areas through optical magnification using an operating microscope. By this way, the ability to find spermatozoa increases compared with the conventional technique (4). Consequently, micro-TESE procedure seems to be the most effective technique with sperm retrieval rates 50-60% in NOA patients (5). There are several factors for prediction of sperm retrieval rates including serum Follicle stimulating hormone (FSH) levels, histopathology, karyotype and testicular size. NOA is usually present with high FSH levels and small testicular volume (6). There are few studies showing the correlations between sperm retrieval rates and testicular size, although evaluating the effectiveness of micro-TESE procedure in NOA patients with severe testicular atrophy.

The aim of this study is to compare the testis sizes with the outcomes of micro-TESE and to evaluate the sperm retrieval success in NOA patients.

Material and Method

This retrospective study has been conducted in the University of Health Sciences, Ankara Dr. Zekai Tahir Burak Women's Health, Health Application, and Research Center following obtaining permission for the research from the institutional review board of the hospital (29.9.2015#61). All participants gave written informed consent before inclusion in the study. This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. The medical records of 80 infertile male patients with a diagnosis of Azoospermia in our institution between January 2016 and May 2017 have been evaluated who have been investigated and treated with Intracytoplasmic sperm injection-Embryo transfer (ICSI-ET) procedures in case of establishment of sperm during TESE procedure.

Risk factors evaluated were; age, duration of infertility, conducting chromosomal karyotype analysis, Y chromosome microdeletion analysis, testicular biopsy examination, basal serum levels of FSH, Luteinizing hormone (LH), total testosterone and measurement of testicular volume before TESE procedures.

Hormonal parameters including FSH, LH, and total testosterone were studied with Electrochemiluminescence Immunoassay "ECLIA" (Roche, E170, Elecsys, Mannheim, Germany) on Elecsys and Cobas immunoassay analyzers in the early morning between hours 08:30-11:30 am.

Azoospermia was diagnosed by centrifugation (3000g) of ejaculate for 15 min with microscopic examination of the pellet with at least two semen samples obtained more than two weeks apart and performed according to the 2010 World Health Organization guidelines (1,7).

Testicular volume was measured with a 7.5 MHz ultrasound probe by the same consultant radiologist and calculated using the formula length (L) x width (W) x height (H) x 0.71 (8). Genetically assessment have been made by using Fluorescence in situ hybridization (FISH) test for detecting the genetic abnormalities.

Female partners of the patients with normal ovarian reserve have received controlled ovarian hyperstimulation (COH) for ICSI-ET. Poor responder female patients were excluded for homogeneity of the study. COH procedures of the patients were commenced with antagonist protocol for all patients. During antagonist protocol, patients have received recombinant FSH starting on days 2 or 3 and 0.25 mg cetrorelix (Cetrotide; Serono, Germany) was administered daily when two or more follicles reached 14 mm in diameter. Human menopausal gonadotropin (hMG) was administered to individual patients when clinically indicated based on the ovarian response to COH treatment. The doses of hMG and recombinant FSH have been adjusted according to the ovarian response for both groups until the day of final oocyte maturation by using hCG. Recombinant hCG (250 micrograms sc., Ovitrelle, Serono, Germany) was administered when at least two leading follicles reached a mean diameter of 17 millimeters. Thirty-six hours after hCG injection transvaginal oocyte retrieval was performed. Following oocyte retrieval, metaphase 2 oocytes were reviewed and day 3 embryo transfer (ET) was performed via using pelvic ultrasonography for all patients. Luteal phase was supported by vaginal progesterone (Crinone 8% gel, Serono, Germany) supplementation twice a day until menstruation or for 12 weeks following the ET procedure in case of a clinical pregnancy establishment. The COH and ICSI-ET outcomes of remaining 43 couples who have been successful for achievement of sperm during TESE evaluated.

Patients divided into two groups in terms of sperm retrieval via TESE. Group 1 defined as patients with positive sperm retrieval and group 2 defined as negative sperm retrieval via TESE.

Microdissection TESE

Microdissection TESE was performed with longitudinal incision and the testicles were delivered out. After opening the tunica vaginalis, tunica albuginea was visualized. After that, an incision was performed to the avascular area of the tunica albuginea under a microscope to uncover the testicular parenchyma at x6-8 magnification. The larger and more opaque seminiferous tubules were extracted at x15-25 magnification. Seminiferous tubules samples were examined by the same embryologist in terms of the presence of spermatozoa. In the absence of spermatozoa from the samples, the procedure was performed to the other testis.

Statistics

Mean and standard deviation (SD) were calculated for continuous variables. The normality of the variables was analyzed by Kolmogorov–Smirnov test. Mann Whitney U test, the chi-square (χ^2) test and Student's t-test were used to evaluate associations between categorical and continuous variables. The receiver operator characteristic (ROC) curve analysis was used to evaluate the predictive role of testicular volume. Logistic regression analysis was performed to find the risk factors of variables for sperm retrieval via TESE by including all related variables to the model. Odds ratio was calculated by a logistic regression method. All variables were included in the backward stepwise procedure. Two-sided p values were considered statistically significant at *p*<0.05. Statistical analyses were carried out by using the statistical packages for SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Sperm extraction has been achieved successfully during TESE among 43 (53%) of 80 patients and 28 of them (35%) achieved embryo availability for transfer. Only one couple had demonstrated fertilization failure following the ICSI procedure. All other patients who have not had embryo transfer was not found to have any sperm during the TESE procedure. Three clinical pregnancies (3.8%) have been achieved following the ICSI-ET procedures of the study group. The karyotypes of two male patients, whose wives are pregnant, were 46, XY and the other one was 46, XXY. Among all azoospermia group, only one patient had Y chromosome microdeletion (AZFb) who has not succeeded to achieve any sperm extraction during TESE. Chromosome analyses of the males have revealed that 5 (6.3%) of the 80 patients had 47, XXY (Klinefelter syndrome) karyotype pattern. Two (40%) of the 5 Klinefelter syndrome (KS) patients have achieved sperm extraction during TESE procedure.

Table I depicted the demographic and clinical characteristics of the patients. There were no statistically significant differences between groups in terms of ages, duration of infertility, smoking, LH levels, total oocyte count, 2PN, embryo count (*p*>0.05). Testicular volume and FSH levels were statistically significantly different between groups (*p*<0.05).

According to the ROC curve analysis (Figure 1) testicular volume was found to be a discriminative parameter in patients with negative retrieval sperm (group 2). The area under curve (AUC), cut of values and sensitivity and specificity are shown in table II. The best testicular volume cut-off value for distinguishing the groups were 6.75 mL with 88.1% sensitivity, 62.1% specificity.

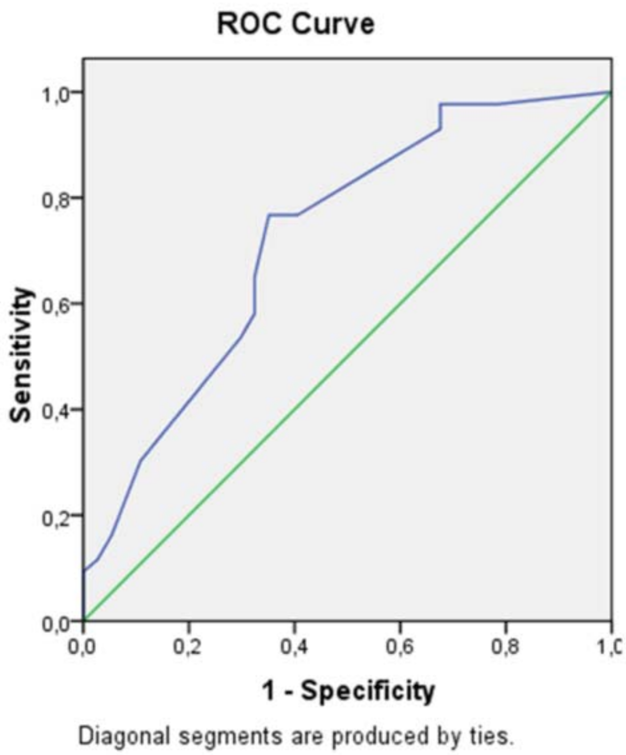

Figure 1: *ROC for the testicular volume (mL) revealing its diagnostic potential for patients with negative sperm retrieval patients.*

Table III summarizes the outcomes of the logistic regression model. According to the model, testicular volume, FSH, total testosterone levels were found to be significant risk fac-

Table I: *Compression of demographic characteristics of groups*

		Group 1 n=43 Mean ± SD	Group 2 n=37 Mean ± SD	*p* value
Male age (year)		31.98±3.37	32.32±4.97	0.724
Female age (year)		28.19±2.94	28.39±4.43	0.810
Duration of infertility (year)		5.71±3.17	5.39±3.55	0.550
Cigarette (n)	Smoke	18	15	0.358
	no smoke	25	22	
Testicular volume (mL)		12.97±4.01	8.54±3.31	<0.001
FSH (mIU/mL)		15.51±9.45	32.28±8.55	<0.001
LH (mIU/mL)		41.48±18.28	24.54±10.31	0.390
Total testosterone (ng/mL)		227.25±161.87	190.43±162.99	0.020
Total oocyte count		9.48±4.75	8.25±2.73	0.128
2PN		3.47±3.18	2.06±0.55	0.358
Embryo count		2.03±2.66	1.82±0.23	0.545

FSH: Follicle stimulating hormone, LH: Luteinizing, 2PN: pronucleus

Table II: *The area under curve, cut of values and sensitivity and specificity for testicular volume in patients*

	AUC	SE	95% CI (Confidence Interval)	Cut of value	Sensitivity (%)-specificity (%)	*p* value
Testicular volume (mL)	.723	.057	.610-.835	6.75	88.1-62.1	<0.001

AUC: Area under curve, SE: Standard error, p<0.05 is statistically significant.

Table III: *Risk factors for negative sperm retrieval via testicular sperm extraction*

	β	SE	Wald	Odds ratio	95% CI	P
Testicular volume	2.33	3.88	4.01	6.39	1.25-26.58	<.001
FSH	1.85	8.91	2.21	1.24	1.11-1.36	<.001
Total testosterone	1.08	161.3	1.13	1.13	0.99-1.21	<.001

TESE: Testicular sperm extraction, FSH: Follicle stimulating hormone, CI: Confidence Interval, SE: Standard error

tors for patients with negative sperm retrieval patients (group 2) (p<0.001). The odds ratios (95% CI) were 6.39 (1.25-26.58), 1.24 (1.11-1.36), 1.13 (0.99-1.21) respectively.

Discussion

Since 1990's, dramatic advances have been produced in the treatment of infertile male patients with NOA. In the past, the options were limited to donor insemination or adoption for this group of patients. The combination of intracytoplasmic sperm injection together with various testicular sperm retrieval techniques gave these men the opportunity to have their own children from their own testis (9).

In our study, the sperm retrieval rate was 53% in 80 patients with non-obstructive azoospermia. In the negative sperm retrieval patients (n=37) FSH levels were high; testicular volume and total testosterone levels found low, significantly. Testicular volume was found to be a discriminative parameter in patients with negative retrieval sperm patients. The cut-off value for distinguishing the groups were 6.75 mL. Testicular volume, FSH, total testosterone levels were found to be significant risk factors for patients with negative sperm retrieval patients.

Testicular sperm extraction (TESE) is an effective method for sperm extraction from patients with NOA. However, TESE is a blind procedure so may fail to identify the isolated focal sperm producing areas. Micro-TESE, is a less invasive procedure and has higher sperm retrieval rates compared with conventional technique (10). Sperm retrieval success was examined with two recent systematic reviews. In one report including seven comparative studies and 1062 patients, the success rate of micro-TESE was higher (42.9 to 63%) than conventional TESE (16.7 - 45%) (11). Similar results with another study, involving 15 studies with a total of 1890 patients, success rate of micro-TESE was 1.5 times higher (95% CI; 1.4-1.6) than the results of TESE (12). Despite small number of patients, the sperm retrieval rate by micro-TESE in patients with NOA was 53% in our study. These findings show that the success of TESE procedure may vary on the different techniques used for sperm retrieval.

There are many studies reporting conflicting evidence that testicular volume can play a role in affecting sperm retrieval rates. Marconi et al. described the "low-chance NOA group" as testicular volumes less than 8 ml and FSH levels as more than 12.4IU with approximately 30% sperm recovery rate (13). Bromage et al. reported that patients with testicular volume less than 4 ml and FSH levels greater than 10 IU had poor sperm retrieval rates with percutaneous epididymal sperm aspiration (PESA) or testicular sperm extraction (TESE) (14). In 2006 Ziaee et al. reported that the cutoff points for successful sperm retrieval were determined as 9.5 mL for testicular volume and 9.9 IU/L for serum FSH (15). These studies lend evidence that smaller testicular size and higher FSH levels has decreased sperm retrieval rates. However Bonarriba et al. showed the relationship between testicular size and sperm retrieval rate but because of small number patients, it was not statistically significant (6). Similarly other authors claimed that there was not a cut-off value of testicular volume for sperm extraction (16-18).

Genetic alterations (Karyotype abnormalities, Y chromosome microdeletion) play an important role in men with NOA. Klinefelter syndrome (47 XXY, 1-2/1000 in male newborns) is the most common karyotype abnormality that causes male hypogonadism and infertility. Spermatogenesis is badly affected with increasing number of X chromosomes (2). Schiff and Okada reported that sperm retrieval rate was 50% in patients with Klinefelter syndrome (19,20). In our study 5 (6.3%) of the 80 patients had 47, XXY (Klinefelter syndrome) karyotype pattern. Only two (40%) of them have achieved sperm extraction during TESE procedure.

Microdeletion of three azoospermia factor regions (AZF a,b,c), located in long arm of Y chromosome (Yq11) and required for normal spermatogenesis, are associated with abnormal spermatogenesis. Studies reported that AZFa, AZFb, AZFb+c microdeletions have not been associated with successful sperm extraction. However AZFc microdeletions are associated with successful sperm extraction in up to 80% rates (21,22). In 2013 Bonarriba et al. similarly found that sperm retrieval rate was 0% in AZFa and AZFb microdeletions. Sperm recovery was 33% in AZFc microdeletion (6). In our study, among all patients with azoospermia, only one patient had Y chromosome microdeletion (AZFb) who has not succeeded to achieve any sperm extraction during TESE.

Conclusion

Micro-TESE is the most effective procedure for patients with NOA. Testicular volume, serum FSH and testosterone levels can be predictive factors for sperm retrieval in men with non-obstructive azoospermia.

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