Effects of clinical, laboratory and pathological features on successful sperm retrieval in non-obstructive azoospermia

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ABSTRACT

Objective: The study aims to evaluate the correlation of testicular sperm extraction (TESE) and histopathology with various features of non-obstructive azoospermia (NOA) cases who consulted to our university-based infertility clinic, and the probability of prompting couples about TESE success and to investigate the cost reduction chance through cost-beneficial aspects.

Material and methods: One hundred and twenty-five patients were enrolled in this study. Age, unprotected intercourse period, age of puberty, and concomitant diseases were noted. Testicular volumes were measured. The correlations between genetic test results and serum levels of Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), free testosterone, prolactin were investigated.

Results: The incidence of NOA among infertile men was found to be 15.1%. Median age of the cases was 33.1 years. Decrease in TESE success rate was seen in the group aged >30, and those who practiced unprotected intercourse for more than 10 years. TESE success rate was 40 percent. The required negative correlation between FSH levels, and testicular volume was not observed when the patient had additional diseases and/or genitourinary surgery. FSH and LH levels were significantly different between TESE- positive and negative groups ($p=0.006$, and $p=0.001$ respectively). Success rate in bilateral TESE group was 14.2%, and 96% of TESE- negative patients had bilateral TESE. Fifteen of 118 patients had Y chromosome microdeletions. These results were similar in both TESE- positive and negative group.

Conclusion: None of the parameters investigated herein predicted successful TESE outcomes. However, in cases with increased FSH and AZFa/AZFb deletion before application of bilateral TESE, in cases of increased FSH and AZFa/AZFb deletion, detailed information should be given to these patients about low success rates and risk of disease inheritance which may reduce procedural costs. Knowing groups with poor prognosis, may help rearrangement of the appropriation of infertility in health policies.

Keywords: Infertility; male factor; non-obstructive azoospermia; TESE.

Introduction

Fifteen percent of infertile males consist of cases with non-obstructive azoospermia (NOA).^[1] Average success rate of testicular sperm extraction was nearly 50 percent.^[2,3] Though in this patient group testicular biopsy has been recommended as the most certain way for predicting prognosis, it is an invasive procedure. Risks of impairment of spermatogenesis, and testicular atrophy exist.^[4] Predictive significance of noninvasive clinical, and laboratory findings before application of invasive methods of testicular sperm extraction (TESE), and intracytoplasmic sperm injection which provide a chance of sperm retrieval has been started to be debated. In various studies performed, poten-

tial predictive significance for post-TESE outcomes have been reported for testicular volume and serum follicle-stimulating hormone (FSH) levels^[5,6], serum inhibin B levels^[5-8], and combination of these two hormones. However as a contrary opinion some studies have also demonstrated that none of these parameters could precisely predict post-TESE outcomes.^[9,10]

In our study the correlations between clinical, laboratory, and demographic characteristics, and TESE outcomes, and testicular pathologies have been investigated. Predictive values of each one of these characteristics in the sperm retrieval of were scrutinized in order to inform the couples about the outcomes of this procedure beforehand.

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Material and methods

Patients with initial diagnosis of male factor infertility between 2002, and 2007 who applied to urology clinic where they received initial diagnosis of NOA were included in the study. Spermogram results of 820 patients were analyzed. Sperm analyses were performed in compliance with the 1999 guideline released by World Health Organization, and semen samples were provided by the patients after masturbation attempted following an abstinence period of 3-6 days. In azoospermic patients, after an at least 4 weeks interval, repeat spermograms were requested to confirm the presence of azoospermia.^[11] In 154 patients, before TESE procedure, in addition to demographic characteristics including age, duration of unprotective sexual intercourse, concomitant diseases that might effect fertility, and previous operations, hormone panel consisting of FSH, luteinizing hormone (LH), prolactin, and free testosterone, testicular volumes, karyotypes, and Y-chromosome microdeletions were analyzed. Non-palpable vas deferens, firm epididymides, ejaculate volume of <2 mL, normal testicular volume, presence of normal FSH, LH, and T levels raised the suspicion of obstructive azoospermia, and these patients were subjected to advanced tests including transrectal ultrasonographic examinations, magnetic resonance imaging, cystic fibrosis gene mutation tests (CFTR), seminal fructose analysis, and then they were excluded from the study. Hundred and twenty-five patients without any missing data were included in the study. Approval of the ethics committee for the conduction of the present study was obtained.

Detailed information about age of the patients, duration of unprotected sexual intercourse, age at onset of puberty, additional diseases which might effect fertility or operations or surgical interventions performed to achieve fertility were obtained.

Orchidometer was used for the measurement of testicular volumes following physical, and urogenital examination.

Venous blood samples drawn from the forearm of the patients between 8 and 10 AM were evaluated. To this end chemoluminometric immune assay method (DPC-Immulate 2000 machine, DPC, Los Angeles, CA, USA) was used. In our laboratory normal FSH (1.3-13.58 mIU/mL), LH (1-8.75 mIU/mL), prolactin (2.5-18.12 ng/mL), and free testosterone (8.6-54.09 pg/mL) levels were also determined.

Genetic analyses of the patients were realized with cytogenetic, and Y chromosome microdeletion analyses. In cytogenetic studies, for chromosomal analysis peripheral blood samples drawn into heparinized tubes were incubated on phytohemagglutinine (PHA) media, and cultivated at 37°C for 72 hours. Forty-five minutes before the study, cells cultivated in colcemid added culture media were lysed in hypotonic solution, and fixed with

Camoy's fixative. The chromosomes retrieved were analysed using GTG banding technique in groups of at least 20 metaphase plaques.

For the analysis of Y chromosome microdeletion, priorly mapped genetic regions (STS: Sequence Tag Sites) on Y chromosome which contained the investigated microdeletions using YDDS™ (Y Deletion Detection Systems, Promega, version 2.0, USA) were amplified with PCR (Polimerase Chain Reaction), and evaluated in 2.5% agarose gel (3/5 agarose + 2/5 NuSieve agarose) under room temperature and 100 V for a duration of 45 minutes. Presence of microdeletions was determined according to the presence or absence of PCR products in electrophoresis. PCR reaction was realized using 20 distinct primer sequences selected among non-polymorphic short DNA segments localized on Y chromosome, and 'multiplex PCR' technique which contains 5 separate PCR reaction mixtures pre-prepared by the manufacturer.

During histopathological examination, the hematoxylin-eosin stained slides were classified based on germ cell aplasia (Sertoli-Cell only), maturation arrest, hypospermatogenesis, tubular necrosis, and normal spermatogenesis. During TESE, histopathology specimens were harvested from the same testicular region of a single or both testes. If spermatozoon was found during TESE of one testicle, then the contralateral testis was not biopsized.

Testicular sperm extraction

After injection of regional anesthetic agent into spermatic cord, median raphe incision was made so as to expose both testes in case of need. In orchiectomized cases only unilateral scrotal incision was made. More voluminous testis palpated on physical examination was preferred. After exposure of the testis, tunica vaginalis was incised, and opened with a 15° microtome under the guidance of an optic loop with 5 X magnification. After necessary hemostatic control, tissue excisions from preferably larger tubuli (if available) were made using iris scissors. The samples were placed in a sperm nutrient solution (G-sperm™, Vitrolife, Gothenburg, Sweden) containing hyaluronic acid, and bicarbonate mixture which is used for assisted reproductive technologies, and the samples were rapidly sent to andrology laboratory to be evaluated by biologists. Firstly tubuli separated by needles were evaluated under light microscope. Then the samples were collected in a conic tube, and materials centrifuged to 40-90% gradients were added. Afterwards, pellets obtained with final centrifugation at 1400 rpm for 10 minutes were re-evaluated, and prepared for fertilization methods. During TESE procedure, one of these tissues was sent for cryopreservation, and the other for histopathological examination. If adequate number of spermatozoa were not found, then the same procedures were repeated till the testicular perfusion was impaired as deemed by the researchers. If any spermatozoa were not found

then the same procedures were applied for the other testis. Tunica albuginea incision line was closed using interrupted 6/0 nylon sutures. While tunica vaginalis was closed with continuous 5/0 vicryl sutures.

Statistical analysis

Statistical evaluation was realized using Statistical Package for the Social Sciences (SPSS Inc; Chicago, IL, USA) 12.0 software program, and $p < 0.05$ was considered to be statistically significant. Data of statistical analysis were presented as mean $\pm$ standard deviation (min- max).

In pairwise intergroup comparisons between testicular volumes, and hormone values, Mann-Whitney U test was used. Data concerning testicular volumes, and hormone values were compared among three groups using Kruskal-Wallis H test. If intergroup differences were found, then Mann-Whitney U test with Bonferroni correction was employed. In the evaluation of data concerning germ cell aplasia (Sertoli Cell-only syndrome: SCOS), hypospermatogenesis, maturation arrest, tubular sclerosis, normal spermatogenesis, Y-chromosome microdeletion, and cytogenetic analysis, *chi-square* or Fisher's exact *chi-square* tests were used.

Based on the presence of previous surgery, concomitant diseases, and karyotypic disorders, correlations among FSH, LH, PRL, free testosterone, testicular volumes, and Y-chromosome microdeletions were compared using Pearson correlation test.

Results

Azoospermia was detected in 154 (18.5%) of 829 patients who applied to our in-vitro fertilisation (IVF) unit between the years 2002, and 2007. When patients with obstructive type azoospermia and/or those couldn't be diagnosed as NOA because of missing data were ruled out, presumptive initial diagnosis of NOA was made in 125 (15.07%) patients.

Mean patient age was 33.16 ± 5.96 (23-47) years, and the patients practiced unprotected sexual intercourse for a mean period of 7.82 ± 5.56 (1.5-25) years. Mean testicular volume, and hormone values of the patients are shown in Table 1.

In our laboratory normal FSH levels were accepted as 1.3-13.58 mIU/mL, while mean FSH level of the patients in our study group was relatively higher (16.00 ± 14.43 (0.1-86.4) mIU/mL). While mean values for LH, prolactin, and free testosterone were within normal limits. Right, and left mean testicular volumes were 12.25 ± 8.16 (0-25) cm³, and 10.20 ± 4.89 (0-20) which were lower than normal values (18-20 cm³).

In 43 of 125 patients, history of genital region surgery was elicited. The patients had previously undergone left varicocele-

lectomy (n=19), bilateral varicocelectomy (n=10), right (n=3), left (n=1) or bilateral orchidopexy (n=3), hydrocelectomy (n=1), right (n=2), and left (n=1) herniorrhaphy, right (n=2), and left (n=2) orchidectomy. NOA patients also had a history of concomitant disease that might effect fertility which included left (n=5), and bilateral (n=1) varicoceles, chemotherapy because of malignancy (n=2), nonspecific orchitis (n=3), mumps orchitis (n=8), testicular trauma (n=3), and posterior pituitary gland dysfunction (n=1).

In 50 (40%) of 125 patients who underwent testicular sperm extraction, sperm was recovered. The correlation between patient's age, and TESE- positivity was evaluated. Though TESE- positivity, and negativity rates were similar in both groups, in patients aged >30 years, a tendency to a decrease in TESE success rates was observed.

In TESE-negative cases FSH, and LH levels were statistically significantly higher relative to TESE-positive cases ($p=0.006$, and $p=0.001$, respectively). A significant difference was not found between groups as for prolactin, free testosterone values, and testicular volumes ($p > 0.05$) (Table 2).

Distribution of TESE results with respect to follicle stimulating hormone levels is shown in Table 3. TESE-positivity was most frequently observed in cases with FSH values between 0, and 10 mIU/mL, while TESE-negativity rate was mostly encountered in the group with FSH levels between 20-30 mIU/mL (94.4%). In cases with various FSH levels TESE results were statistically significantly different ($X^2=14.303$, $p=0.003$).

Testicular tissues of the patients who underwent bilateral TESE procedures were sent separately for histopathological examination, with indication of the biopsied sides. In unilateral procedures or in orchidectomized cases only one specimen was sent to the laboratory for histopathological examination. Distribution of histopathological results of right, and left testes were almost equal. Correlation between the histopathological results of the right testis with TESE results is shown in Table 4. In the hypospermatogenetic group TESE positivity was seen in 17 of 27 (62.9%) patients.

When all cases with non-obstructive azoospermia were evaluated, most frequently SCOS was encountered (right testis 46.4%, and left testis 50%), followed by hypospermatogenesis, maturation arrest, and tubular sclerosis in decreasing order of frequency.

When cases were evaluated as for TESE- positivity, or negativity, hypospermatogenesis was the most frequently observed pathology in TESE- positive cases followed by SCOS, maturation arrest, tubular sclerosis, and normal spermatogenesis in the right

Table 1. Testicular volumes, and Hormone Values [Mean $\pm$ SD (Min-Max)]

Group N (n=125)	
FSH (mIU/mL)	16.00 $\pm$ 14.43 (0.1-86.4)
LH (mIU/mL)	6.97 $\pm$ 5.48 (0.7-35.6)
PRL (ng/mL)	10.32 $\pm$ 6.59 (2.9-52.7)
Free testosterone (pg/mL)	12.25 $\pm$ 8.16 (8.6-54.09)
Right testicular volume (cm ³)	12.25 $\pm$ 8.16 (0.0-25.0)
Left testicular volume (cm ³)	10.20 $\pm$ 4.89 (0.0-20.0)
FSH: follicle stimulating hormone; LH: luteinizing hormone; PRL: prolactin; SD: standard deviation	

Table 2. Comparison of hormone levels, and testicular volumes within TESE-positive, and negative cases [Mean $\pm$ SD (Min-Max)]

	TESE-positive (n=50)	TESE-negative (n=75)	p
FSH (mIU/mL)	11.72 $\pm$ 10.94 (0.1-60.0)	18.85 $\pm$ 15.78* (2.4-86.4)	0.006
LH (mIU/mL)	5.02 $\pm$ 3.06 (0.7-15.0)	8.27 $\pm$ 6.32* (1.1-35.6)	0.001
PRL (ng/mL)	9.44 $\pm$ 4.93 (2.9-27.0)	10.90 $\pm$ 7.47 (3.8-52.7)	0.228
Free testosterone (pg/mL)	13.22 $\pm$ 9.70 (8.6-54.09)	11.59 $\pm$ 6.91 (3.2-47.3)	0.280
Right testicular volume (cm ³)	10.24 $\pm$ 5.78 (0.0-24.0)	10.43 $\pm$ 5.36 (0.0-25.0)	0.849
Left testicular volume (cm ³)	10.63 $\pm$ 5.07 (0.5-20.0)	9.91 $\pm$ 4.78 (0.0-20.0)	0.425
*p<0.05: When compared with TESE-positive group. FSH: follicle stimulating hormone; LH: luteinizing hormone; PRL: prolactin; TESE: testicular sperm extraction			

Table 3. Distribution of TESE- positive, and negative cases based on FSH levels [n, (%)]

	FSH (0-10) (n=49)	FSH (>10-20) (n=45)	FSH (>20-30) (n=18)	FSH (>30) (n=13)
TESE (+)	26 (53.1)	20 (44.4)	1 (5.6)	3 (23.1)
TESE (-)	23 (46.9)	25 (55.6)	17 (94.4)	10 (76.9)
TESE: testicular sperm extraction; FSH: follicle stimulating hormone				

Table 4. Correlation between histopathologic examination results of the right testis, and TESE results [n, (%)]

	TESE-positive (n=36)	TESE-negative (n=74)	p
SCOS	8 (22.2)	43 (58.1)	X ² =22.188
Hypospermatogenesis	17 (47.2)	10 (13.5)	
Maturation arrest	7 (19.4)	17 (23)	<0.0001
Tubular sclerosis	2 (5.6)	4 (5.4)	
Normal spermatogenesis	2 (5.6)	0 (0)	
SCOS: Sertoli-Cell Only syndrome; TESE: testicular sperm extraction			

testis, and in the left testis maturation arrest, SCOS, and tubular sclerosis were encountered in decreasing order of frequency. A statistically significant difference was found between TESE-positive, and negative groups as for distribution of histopathology results (p<0.0001).

In assessments performed separately for each testis, when compared with the SCOS group, FSH, and LH levels in groups of hypospermatogenesis, and maturation arrest were markedly lower (p<0.05). Testicular volumes, free testosterone, and PRL levels were similar between groups.

In intragroup comparisons between tubular necrosis seen in the right testes of 6 cases, and maturation arrest were compared, significant increase in LH level was seen in the tubular necrosis group (p=0.002), while a marked decrease in testicular volumes in the tubular necrosis group (p=0.038) was detected. Though not statistically significant, higher FSH levels were detected in the maturation arrest group.

In the comparison between pathologic groups of tubular necrosis, and maturation arrest, higher LH levels were found in the left testicular tubular necrosis group (p=0.013). Even though any statistically significant difference was not found among left testicular volumes, lower testicular volumes were seen in the tubular necrosis group.

Histopathological examinations were performed from a single testis in 41 (32.8%) patients if the patients were orchiectomized or spermatozoa were detected in only one testis, while in 84 (67.2%) of the patients the samples were obtained from both testes.

In TESE-negative cases, histopathology specimens were mostly (n=72; 96%) retrieved from both testes, while in TESE-positive cases specimens were retrieved mostly (n=38 76%) from a single testis. Histopathological examination results were statistically

Table 5. Histopathologicla examination data of testicles [n, (%)]

	TESE- positive (n=50)	TESE- negative (n=75)	p
Unilateral histopathology specimen	38 (76)	3 (4)	X ² =70.557
Bilateral histopathology specimen	12 (24)	72 (96)	
			<0.0001
TESE: testicular sperm extraction			

Table 6. Karyotypic examination data [n, (%)]

	TESE- positive (n=49)	TESE- negative (n=69)	p
46XY	49 (100)	64 (92.8)	X ² =3.708
47XXY	-	2 (2.9)	
46XY 22p+	-	1 (1.4)	0.447
46XY 13 p-	-	1 (1.4)	
46 XY t(16;22) (13,3;q12)	-	1 (1.4)	
TESE: testicular sperm extraction			

Table 7. Y chromosome microdeletion data [n, (%)]

	TESE- positive (n=50)	TESE- negative (n=75)	p
Normal	46 (93.9)	57 (82.6)	X ² =3.691 0.449
AZF a	-	1 (1.4)	
AZFb	1 (2)	4 (5.8)	
AZFc	2 (4.1)	6 (8.7)	
AZFb+AZFc	-	1 (1.4)	
AZF: Azoospermia factor; TESE: testicular sperm extraction			

significantly different between TESE- negative, and TESE- positive cases ($X^2=70.557$, $p<0.0001$), (Table 5). TESE- positivity rates were detected in 92.6% of 41 patients with unilateral, 84 (14.2%) cases with bilateral biopsy results.

In 13 (15.8%) of 82 cases with bilateral histopathology results, right, and left testicular histopathology results were different. Viable spermatozoa were found in only 2 patients who had undergone TESE procedures. In 7 (10.1%) of 69 patients with bilaterally similar histopathology TESE- positivity was encountered.

In groups with different or similar bilateral testicular biopsy results, the number of TESE- positive and negative cases was nearly similar.

Karyotype analyses, and comparative values are shown in Table 6. Y chromosome microdilution was detected in 15 of 118 patients, and relevant data are given in Table 7. Distribution of the cases was as follows: fertile (n=103; 87.3%), AZFa (n=1; 0.8%), AZFb (n=5; 4.2%), and AZFc (n=8; 6.8%), AZFb+AZFc (n=1; 0.8%). Y-chromosome microdilution data of TESE- negative, and TESE- positive values were nearly similar ($X^2=3.691$, $p=0.449$) (Table 7).

Discussion

In various studies performed, an average sperm retrieval rate of 59% has been reported using TESE.^[12] In these studies a precise parametre which can predict sperm recovery has not been determined. Though studies have been conducted on various parametres including testicular pathology, levels of FSH, and inhibin, testicular volumes, genetic variables, diagnostic testicular biopsy (DTB) has been the prognostic marker with the highest predictive value. Presence of mature spermatids has been defined as the best marker for the presence of mature spermatozoa.^[13,14]

Apart from diagnostic testicular biopsy two of the mostly emphasized parametres have been FSH, and testicular volumes. Increased FSH levels, and small testicular volumes have been associated with testicular failure. An inverse correlation between FSH levels, and number of spermatogonia has been demonstrated.^[15,16] Even though this inverse proportion is a very well known fact, either FSH or the recently emphasized inhibin does not demonstrate a strictly inverse proportion with spermatogenesis, and either of them is not a robust marker predicting sperm retrievability.^[14] In nearly one third of the patients with spermatogenetic defect, normal FSH levels were detected. Defective spermatogenesis can be observed in patients with normal FSH levels, and as a known fact in the presence of very high FSH levels, motile spermatozoa can be retrieved.^[17] Also in our study, in 2 patients with FSH levels of 41.6, and 60 mIU/mL, respectively, sperm retrieval could be achieved during TESE. In a study by Schwarzer et al.^[17] in the year 2003, 414 NOA patients were grouped according to FSH levels of 0-10, 10-20, 21-30, 31-40 ve 40 mIU/mL, and a statistically significant TESE-positivity was not detected between groups. However in our study when FSH levels of patients were grouped as 0-10, 11-20, 21-30, and >30 mIU/mL, a statistically significant difference was detected between groups. The highest sperm recovery rate was encountered in group with FSH levels between 0, and 10 mIU/mL, while higher sperm retrieval rate in the FSH group with >30 mIU/mL relative to 20-30 mIU/mL FSH group, confirms the information which asserts that FSH values are not

very strict predictive markers for sperm retrieval rates. Besides, the patients can be informed about their chances of successful sperm retrieval based on their FSH levels. TESE-negativity rates of 94.4% seen in patients with FSH values between 20, and 30 mIU/mL, may bring cryopreservation on to agenda. In this way unnecessary applications of ovarian stimulation may be curtailed. In our study the reason why FSH is not a strict predictive marker for sperm retrieval is that successful sperm retrievals can be achieved in patients FSH levels over 30 mIU/mL.

LH levels can change in patients with non-obstructive azoospermia. In isolated spermatogenic failure LH levels do not change, while in testicular failure, increases in both LH, and FSH levels can be observed.^[18] Based on the data we obtained in our study although average LH levels of 125 patients diagnosed as NOA were within normal limits, a positive correlation was found between levels of LH, and FSH. Based on the knowledge that higher LH levels can be observed in cases apart from isolated spermatogenic failure, when patients were evaluated according to the presence (if any) of concomitant disease(s) or surgical operations which may effect testicular perfusion, a positive correlation was also observed between FSH, and LH levels in all groups. Besides when patients were divided into two groups as TESE-positive, and TESE-negative LH levels in the TESE-negative group were found to be significantly higher versus TESE-positive group. When patients were evaluated based on histopathology results of right, and left testes, still intergroup differences were observed as for LH levels. LH levels of SCOS group were found to be prominently higher when compared with those of hypospermatogenesis, and maturation arrest groups. Highest LH levels were detected in the tubular sclerosis group highest LH levels were detected. Similarly LH levels were within normal limits in the maturation arrest, and hypospermatogenesis groups, while they were still significantly lower when compared with those of the SCOS group. LH levels of NOA patients were evaluated. In TESE- negative cases LH values were within the upper limit of normal or supranormal levels, while in TESE-positive cases they were within normal limits. In the light of these data, and as far as we know, variations in LH levels which do not provide any evidence suggesting sperm retrieval capacity of TESE, are observed when they are evaluated among NOA patients regarding histopathological subgroups or development of testicular insufficiency (if any) due to causes apart from pituitary disorders.

Based on our data, karyotypic and Y-chromosome anomalies can be seen in all cases with various testicular volumes of all cases which indicate that genetic analyses should be performed separately. When patients were evaluated based on TESE outcomes, inability to detect any significant difference between testicular volumes suggests us that TESE procedures can be performed even in very small testes. Indeed, in our study TESE-positivity was seen in 8 of 17 patients whose testicular volumes were less than 5 mL.

Among NOA patients who had undergone testicular sperm extraction, TESE success rates vary according to pathologic subgroups. From this perspective the best results were obtained in the hypospermatogenesis group. Tournaye et al.^[19] achieved sperm retrieval in all patients in the hypospermatogenesis group, Su et al.^[13] achieved sperm retrieval in 79% of the cases with hypospermatogenesis, then in cases with maturation arrest, and SCOS in decreasing order of frequency. In our study, sperm retrieval rates in these histopathological groups were similar to those reported in the literature, while in our study combined TESE success rate including both right, and left testes was 60.7 percent. The most possible cause of this lower success rate might be related to inability to perform TESE using microsurgery. A statistically significant difference was not detected between histopathological evaluation results of right, and left testis *per se*. Evaluation of the right testis demonstrated SCOS (n=51; 46.4%), hypospermatogenesis (n=27; 24.5%), maturation arrest (n=24; 21.8%), tubular sclerosis (n=6; 5.5%), and normal spermatogenesis (n=2; 1.8%) in respective number of patients. Histopathology of 2 cases evaluated as NOA at the start of the study, was reported as normal spermatogenesis which revealed the margin of error in the diagnosis of NOA, but it might also suggest obstruction of rete testis which can be hardly discriminated from obstructive type azoospermia. When TESE results for the right testis were categorized in histopathological groups, most frequently observed abnormalities in the TESE-positive, and negative groups were hypospermatogenesis, and SCOS, respectively. In both testes combined TESE-positivity rates in the groups of SCOS, and hypospermatogenesis were found as 15.6, and 62.9%, respectively which were lower than those reported in the literature. However statistically significant differences were found between histopathological subgroups as for TESE positivity.

Median rate of TESE- positivity was 92.6%, when unilateral testicular sperm extraction provided sufficient spermatozoa, while in patients who underwent bilateral testicular biopsies TESE-positivity was only 14.2 percent. If histopathology report indicated the presence of SCOS in both testes, this rate dropped to 10.1 percent. According to our available data, up to now, this difference was not revealed in unilateral, and bilateral histopathological analyses. However, this important difference demonstrates that the possibility of sperm positivity is greater before repetitive cycles in NOA patients who had previously undergone unilateral TESE. Similarly, the patients who had previously undergone bilateral TESE can be informed about the lower possibility of sperm retrieval. Dramatically lower TESE success rates have been seen in patients whose histopathological examination of bilateral biopsy specimens were reported as SCOS. Besides, specimens harvested from these patients with lower TESE success rates can be cryopreserved so as to be able to refrain from unnecessary ovarian stimulation procedure with resultant decrease in expenditures.

In addition to differences in FSH levels between pathological subgroups, LH levels also differed significantly between these subgroups.

In comparisons among Sertoli cell- only, hypospermatogenesis, and maturation arrest groups, significantly lower FSH levels were observed in the last two groups, however FSH levels were within normal limits in the hypospermatogenesis group. Similar data were also reported for LH levels which previously had not demonstrated differences among groups.^[20,21] In our study, LH levels displayed significant differences between TESE- positive, and TESE-negative patients, and histopathological subgroups. These findings indicate the necessity of conduction of further studies aiming to enlighten the differences between LH levels detected among histopathological subgroups during evaluations within NOA patient groups. In addition, when groups of tubular necrosis, and maturation arrest were compared, markedly lower FSH, LH levels, and testicular volumes in the tubular sclerosis group were detected. In the patient group with tubular necrosis, very high FSH, and LH levels, and lower testicular volumes were related to characteristic pathologic findings secondary to end-stage testicular development. In 2 of 6 patient, non-mosaic Klinefelter syndrome was detected, and TESE –negative results were obtained in these patients.

Increase in LH levels, and decrease in testicular volume can predict the outcome of such a pathology more predominantly especially in a patient with relevant clinical markers seen in the tubular sclerosis group when compared with other groups. However as indicated in the literature because of TESE- positivity indicated in the literature, it does not appear to have any practical benefit.

In the evaluation of demographic data, patients' age, duration of unprotected sexual intercourse, and age of onset at puberty were similar between TESE- negative, and positive groups. However in older patients, and patients who practiced unprotected sexual intercourse for a long time, remarkably lower rates of successful outcomes could be achieved. Therefore since with aging concomitant diseases will emerge which might adversely effect testicular perfusion, and germ cell loss, it will be reasonable to initiate treatment of infertile couples at an optimal time.

Inverse correlation between FSH, and testicular volume observed in the group without any concomitant disease was not encountered among patients with concomitant diseases including varicocele, mumps orchitis, trauma or testicular torsion which might effect their fertility potential adversely. This difference might stem from inconvenience of orchidometric measurement method used in this patient group. Use of ultrasound in this patient group may lead to achievement of more accurate results. Indeed, ultrasonographic estimation of testicular volumes in all of NOA patients, and sonographic mapping using a colour-Doppler

US during this procedure may detect regions with improved perfusion. A previous study demonstrated that this method yielded more improved TESE outcomes.^[22]

In 118 of 125 patients who applied to our clinic with infertility, and diagnosed as NOA, karyotype analysis, and Y-chromosome microdeletions were studied, and nonmosaic Klinefelter syndrome was detected in 2 patients. Sperm retrieval rates of 40-48% were reported in large series.^[23-25] In a large series performed by Okada et al.^[26] only patient's age was demonstrated as the only parametre effecting TESE outcomes. Median ages in failed, and successful TESE interventions were found to be 38 (28-43), and 31 (25-40) years, respectively. Indeed, during our follow-up period one of our patients was 38, and the other 42 years of age. In the light of these data, the information indicating rapid decrease in germ cells with age in Klinefelter syndrome is confirmed.^[18,27] Apart from this, autosomal translocations were encountered in 3 patients with karyotypic disorders which consisted of 4.2% of the cases with NOA. Sperm retrieval attempts failed in all of our patients with karyotypic disorders.

We detected Y chromosome microdeletions in 12.7% of the cases. In the literature these rates change between 8, and 18 percent.^[28-32] Apart from normal Y chromosome configuration, most frequently AZFc gene deletions were observed in 8 (6.7%) cases. However AZFc, AZFb (n=5), AZFa (n=1), and AZFb+AZFc (n=1) gene deletions were also detected in respective number of patients. It is known that the best TESE outcomes have been obtained in the AZFc group.^[33] In compliance with these information, also in our study, in 2 (33.3%) out of 6 patients, spermatozoa could be retrieved using TESE. As is recognized, in complete deletions in the regions of AZFa, and AZFb, retrieval of spermatozoa is a very low possibility. In a review of another 10-year-old series, AZFc deletions were encountered in 66% of all cases with Y-chromosome microdeletions.^[34] In our study in 8 (53.3%) of 15 patients AZFc deletions were detected, and sperm retrieval could not be achieved in patients with AZFa, and AZFb+AZFc gene deletions. In the literature a successful sperm retrieval has not been reported in cases with complete AZFb deletions. Though data indicating sperm retrieval in cases with partial AZFb deletions are available, spermatozoa can be even encountered in ejaculates of the patients who applied because of infertility. Besides AZFb gene deletion was encountered in spontaneously delivered 3 male children of a father with this deletion. AZFb deletion was complete in 1, and partial in the other 2 children.^[33-35] In our study, AZFb (RBM1) partial deletion was encountered in one of 5 patients with AZFb gene deletion. In the light of all these data prognostic value of partial or complete AZFb deletion differs. Indeed, in cases with complete deletion, sperm retrieval has not been successful so far. Still, also in partial deletions sperm retrieval rates may be too low. Therefore in cases with complete AZFb gene deletion Brandell et al.^[31] demonstrated predictive value of complete AZFb gene dele-

tion which can be further supported. In addition of all these, patients with Y-chromosome microdeletion were investigated both from clinical, and phenotypical aspects, their serum FSH levels, and testicular volumes were analyzed, and histopathological results were evaluated. FSH, LH, testosterone levels, and testicular volumes did not differ between these patients with and without Yq deletion.^[34] However significantly lower FSH levels were reported in the subgroup of AZFb gene deletion relative to other subgroups, which were not in compliance with our data. However in one of our patient in the AZFb group whose histopathologic examination of both testis biopsies were interpreted as bilateral tubular necrosis, detection of lower FSH (3.3 mIU/mL), and LH (5.3 mIU/mL) levels when compared with other patients with tubular necrosis can be considered as an issue which requires further investigation. In these patients testicular volumes show differences between 5, and 20 cc which were independent from study outcomes, and FSH levels demonstrate that in advanced researches performed for NOA, genetic factor should not be overlooked.

The cases apart from AZFc which have been observed in 50-66% of the cases with non-obstructive azoospermia, the probability of successful sperm retrieval is almost none.^[36] In addition to all of these factors, when low success rate of TESE-ICSI and genetic transmission of this disorder to their offsprings are told to the couples properly, then up to 20% of these couples can refrain from undergoing this procedure.^[37,38] In the light of these data, when clinical characteristics of the patients were evaluated in detail together with genetic counselling, one can refrain from very costly TESE-ICSI procedures or repetition of these procedures in case of previous failure.

Our study have demonstrated that none of the parametres examined is not a clear-cut predictive marker for sperm retrieval, however they can provide important clues. Before recurrent cycles, significant data can be given to clinicians about cost-effectiveness of the procedure, and request them to enlighten their patients about this issue.

Since in patients who had undergone bilateral TESE surgeries, the success rate of TESE is very low (14.2%), before repeat TESE-ICSI procedures, the patients should be told about this low rate so as to decrease unnecessary costs, and discourage patients from undergoing cryopreservation to induce ovarian stimulation.

In one of our patients with FSH level of 60 mIU/mL, a successful sperm retrieval was achieved. Therefore the patient should not be provided with information based on seemingly absolute judgement after analyzing FSH levels. However in our study our success rate with TESE in patients with FSH levels ranging between 20-30 mIU/mL was only 5.6% Therefore, in consideration of difficulties in affording these treatment modalities, enlightened information should be given to the patients.

In studies performed with non-obstructive patient group, up to now generally only statistically insignificant data have been obtained about LH. However based on our information LH demonstrated differences between both TESE- positive, and negative patients, and more importantly between histopathological subgroups. Thanks to this difference between histopathological subgroups, clinicians can evaluate the outcomes of testicular biopsy more precisely before TESE procedure (if applied), and for example suspect the presence of mixed type histopathology based on the differences between LH levels which urge them to perform testicular mapping either by using multiple testicular biopsies or Doppler ultrasound leading to more improved outcomes.

In the presence of concomitant diseases, and previous surgery, hormone profiles, pathology outcomes, and testicular volumes should be evaluated more attentively. Inverse proportion between FSH, and testicular volume which is accepted to be one of the general characteristics of NOA patient group was not encountered in our patient group. In these patients rather than testicular volume measurements with orchidometer, use of a more objective method ie. Doppler US may ensure more accurate volume estimates, and increase the success rate of TESE providing a better chance of selecting more effectively perfused areas.

In patients with AZFa, and AZFb deletions in whom any fertile spermatozoa could not be retrieved, hormone profiles, testicular volumes, and more importantly testicular histologies which are considered to be the most accurate prognostic markers do not demonstrate marked differences from other patients with NOA. In this group of patients TESE is not successful at all, and this disorder which can be genetically transmitted to offsprings should be explained to the couples in full detail, and so costly procedures, and repetitive interventions can be avoided.

In conclusion none of the parametres examined in patient groups with NOA could precisely predict sperm retrieval. However if detailed information is given to the patients who had previously undergone bilateral procedures, about higher FSH levels, clinical conditions as AZFa, and AZFb deletions, about the risk of inheritance, and lower success rates, then the patients can possibly refrain from unnecessary procedures or ovarian hyperstimulations with resultant decrease in the cost of the procedures, and drugs used.

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References

1. Dubin L, Amelar RD. Etiologic factors in 1294 consecutive cases of male infertility. *Fertil Steril* 1971;22:469-74. [CrossRef]
2. Palermo GD, Schlegel PN, Hariprasad JJ, Ergün B, Mielnik A, Zaninovic N, et al. Fertilization and pregnancy outcome with intracytoplasmic sperm injection for azoospermic men. *Hum Reprod* 1999;14:741-8. [CrossRef]
3. Turek PJ, Givens CR, Schriock ED, Meng MV, Pedersen RA, Conaghan J. Testis sperm extraction and intracytoplasmic sperm injection guided by prior fine needle aspiration mapping in the non-obstructive azoospermia. *Fertil Steril* 1999;71:522-5. [CrossRef]
4. Schlegel PN, Su LM. Physiological consequences of testicular sperm extraction. *Hum Reprod* 1997;12:1688-92. [CrossRef]
5. Bohring C, Schroeder-Printzen I, Weidner W, Krause W. Serum levels of inhibin B and follicle-stimulating hormone may predict successful sperm retrieval in men with azoospermia who are undergoing testicular sperm extraction. *Fertil Steril* 2002;78:1195-8. [CrossRef]
6. Goulis DG, Polychronou P, Mikos T, Grimbizis G, Gerou S, Pavlidou V, et al. Serum inhibin-B and follicle stimulating hormone as predictors of the presence of sperm in testicular fine needle aspirate in men with azoospermia. *Hormones (Athens)* 2008;7:140-7.
7. Nagata Y, Fujita K, Banzai J, Kojima Y, Kasima K, Suzuki M, et al. Seminal plasma inhibin-B level is a useful predictor of the success of conventional testicular sperm extraction in patients with non-obstructive azoospermia. *J Obstet Gynaecol Res* 2005;31:384-8. [CrossRef]
8. Chen SC, Hsieh JT, Yu HJ, Chang HC. Appropriate cut-off value for follicle-stimulating hormone in azoospermia to predict spermatogenesis. *Reprod Biol Endocrinol* 2010;8:108. [CrossRef]
9. Tunc L, Kirac M, Gurocak S, Yucel A, Kupeli B, Alkibay T, et al. Can serum Inhibin B and FSH levels, testicular histology and volume predict the outcome of testicular sperm extraction in patients with non-obstructive azoospermia? *Int Urol Nephrol* 2006;38:629-35.
10. Carpi A, Sabanegh E, Mechanick J. Controversies in the management of nonobstructive azoospermia. *Fertil Steril* 2009;91:963-70. [CrossRef]
11. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction. 4th ed. Cambridge: Cambridge University Press; 1999.
12. Schlegel PN. Sperm retrieval techniques for assisted reproduction. In Werthman PE (eds): *Infertility and Reproductive Medicine Clinics of North America*. W.B Saunders company 1999;10:539-55.
13. Su LM, Palermo GD, Goldstein M. Testicular sperm extraction with intracytoplasmic sperm injection: Preoperative histology can predict success of sperm retrieval. *J Urol* 1999;161:112-30. [CrossRef]
14. Tournaye H, Verheyen G, Nagy P, Ubaldi F, Goossens A, Silber S, et al. Are there any predictive factors for successful testicular sperm recovery in azoospermic patients? *Hum Reprod* 1997;12:80-6.
15. Ishikawa T, Fujioka H, Fujisawa M. Clinical and hormonal findings in testicular maturation arrest. *BJU Int* 2004;94:1314-6. [CrossRef]
16. de Kretser DM, Burger HG, Hudson B. The relationship between germinal cells and serum FSH levels in males with infertility. *J Clin Endocrinol Metab* 1974;38:787-93. [CrossRef]
17. Schwarzer JU, Fiedler K, Hertwig IV, Krüsmann G, Würfel W, Mühlen B, et al. Male factors determining the outcome of intracytoplasmic sperm injection with epididymal and testicular spermatozoa. *Andrologia* 2003;35:220-6. [CrossRef]
18. Sigman M, Jarow JP. Male infertility. *Cambell's Urology* 10th ed, section VI, 2012: 627-58.
19. Tournaye H, Liu J, Nagy PZ. Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular spermatozoa. *Hum Reprod* 1996;11:127-30. [CrossRef]
20. Bar-On E, Weiss DB, Gottschalk-Sabag S, Zukerman Z. The relationship between plasma levels of gonadotropins, androgens, and prolactin in azoospermic men with testicular spermatogenic arrest pattern. *Fertil Steril* 1995;64:1043-5. [CrossRef]
21. Ezech UIO, Moore HD, Cooke ID. Correlation of testicular sperm extraction with morphological, biophysical and endocrine profiles in men with azoospermia due to primary gonadal failure. *Hum Reprod* 1998;11:3066-74. [CrossRef]
22. Tunç L, Alkibay T, Küpeli B, Tokgöz H, Bozkirli I, Yücel C. Power Doppler Ultrasound mapping in nonobstructive azoospermic patients prior to testicular sperm extraction. *Arch Androl* 2005;51:277-83. [CrossRef]
23. Friedler S, Raziel A, Strassburger D, Schachter M, Bern O, Ron-El R. Outcome of ICSI using fresh and cryopreserved-thawed testicular spermatozoa in patients with non-mosaic Klinefelter's Syndrome. *Hum Reprod* 2001;16:2616-20. [CrossRef]
24. Levron J, Aviram-Goldring A, Madgar I, Raviv G, Barkai G, Dor J. Sperm chromosome analysis and outcome of IVF in patients with non-mosaic Klinefelter's Syndrome. *Fertil Steril* 2000;74:925-9. [CrossRef]
25. Vernaev V, Staessen C, Verheyen G, Van Steirteghem A, Devroey P, Tournaye H. Can biological or clinical parameters predict testicular sperm recovery in 47,XXY Klinefelter's Syndrome patients? *Hum Reprod* 2004;19:1135-9.

26. Okada H, Goda K, Yamamoto Y, Sofikitis N, Miyagawa I, Mio Y, et al. Age as a limiting factor for successful sperm retrieval in patients with nonmosaic Klinefelter's syndrome. *Fertil Steril* 2005;84:1662-4. [\[CrossRef\]](#)
27. Gorddon DL, Krmpotic E, Thomas W, Gandy HM, Paulsen CA. Pathologic testicular findings in Klinefelter's Syndrome. 47,XXY vs 46,XY-47,XXY. *Arch Intern Med* 1972;130:726-9. [\[CrossRef\]](#)
28. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet* 1996;5:933-43. [\[CrossRef\]](#)
29. Reijo R, Alagappan R, Patrizio P, Page DC. Severe oligospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet* 1996;347:1290-3. [\[CrossRef\]](#)
30. Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, et al. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nature Gen* 1995;10:383-93. [\[CrossRef\]](#)
31. Brandell RA, Mielnik A, Liotta D, Ye Zi Veeck LL, Palermo GD, Schlegel PN. AZFb deletions predict the absence of spermatozoa with testicular sperm extraction: preliminary report of a prognostic genetic test. *Hum Reprod* 1998;13:2812-5. [\[CrossRef\]](#)
32. Martinez MC, Bernabe MJ, Gomez E, Ballesteros A, Landeras J, Glover G, et al. Screening for AZF deletion in a large series of severely impaired spermatogenesis patients. *J Androl* 2000;21:651-5.
33. Choi JM, Chung P, Veeck L, Mielnik A, Palermo GD, Schlegel PN. AZF microdeletions of the Y chromosome and in vitro fertilization outcome. *Fertil Steril* 2004;81:337-41. [\[CrossRef\]](#)
34. Ferlin A, Arredi B, Speltra E, Cazzadore C, Selice R, Garolla A, et al. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. *J Clin Endocrinol Metab* 2007;92:762-70. [\[CrossRef\]](#)
35. Samli H, Murat Samli M, Solak M. Natural transmission of AZFb Y-chromosomal microdeletion from father to his three sons. *Arch Androl* 2006;52:423-6. [\[CrossRef\]](#)
36. Foresta C, Moro E, Ferlin A. Prognostic value of Y deletion analysis. *Hum Reprod* 2001;16:1543-7. [\[CrossRef\]](#)
37. Nap AW, Van Golde RJ, Tuerlings JH, De Sutter P, Pieters MH, Giltay JC, et al. Reproductive decisions of men with microdeletions of the Y chromosome: the role of genetic counselling. *Hum Reprod* 1999;14:2166-9. [\[CrossRef\]](#)
38. Rucker GB, Mielnik A, King P, Goldstein M, Schlegel PN. Pre-operative screening for genetic abnormalities in men with non-obstructive azoospermia before testicular sperm extraction. *J Urol* 1998;160:2068-71. [\[CrossRef\]](#)