Obstet Gynecol Res 2021; 4 (1): 012-020 DOI: 10.26502/ogr050

Research Article

Ethnic Origin as a Parameter in the Prediction of Successful Testicular Sperm Extraction in Patients with Non-Obstructive Azoospermia

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Received: 19 January 2021; **Accepted:** 27 January 2021; **Published:** 29 January 2021

Citation: Atif Zeadna, Iris Har-Vardi, Naheel Khateeb, Naama Steiner, Arie Berkovitz, Yotam Lior, Etan Levitas, Eitan Lunenfeld, Eliahu Levitas. Ethnic Origin as a Parameter in the Prediction of Successful Testicular Sperm Extraction in Patients with Non-Obstructive Azoospermia. Obstetrics and Gynecology Research 4 (2021): 012-020.

Abstract

Background: The need for a reliable prediction model of testicular sperm extraction outcomes in non-obstructive azoospermia patients is inarguably of paramount importance. The aim of the present study was to determine whether a combination of available parameters would improve the prediction of successful sperm retrieval.

Methods: A retrospective cohort study with a population of 119 patients who underwent Testicular Sperm Extraction - was conducted. We used univariate and multivariate binary logistic regression to identify the most reliable factors associated with the success of the surgical procedure.

Results: Age and ethnicity were the only parameters that differed significantly between the patients who experienced successful testicular sperm extraction and those who did not. A comparison between patients of Jewish and Bedouin origin demonstrated that despite the fact that the Jewish population was significantly older,

the sperm retrieval rate was much higher than it was in the Bedouin population-77.19% versus 54.88%.

Conclusion: To the best of our knowledge, this is the first study to emphasize ethnic origin as an important parameter in the prediction of successful sperm retrieval in non-obstructive azoospermia patients.

Keywords: Male Infertility; Ethnic Origin; Non-Obstructive Azoospermia; Sperm Retrieval

1. Introduction

Infertility is a common disorder among young men and women, affecting approximately 15% of all couples. Causes of male infertility include genetic factors. One of 20 infertile men bears a chromosomal abnormality. Eighty percent of genetic cases involve sex chromosomes and 20% involve autosomal abnormalities. Klinefelter syndrome is the chromosomal disorder most frequently associated with non-obstructive azoospermia. In addition, Y chromosome deletions have been linked with cryptozoospermia and azoospermia, emphasizing the importance of the Y chromosome with its collection of testis-specific genes necessary for spermatogenesis. Furthermore, infectious factors, endocrine problems, and iatrogenic factors (following treatment such as radiotherapy and/or chemotherapy in cancer patients) may be involved in sperm disorders, while most cases are idiopathic [1, 2]. Azoospermia is diagnosed when no spermatozoa are detected using high-powered microscope examination of centrifuged seminal fluid on at least two occasions. It is present in approximately 10% to 15% of all investigated infertile men. The etiology of azoospermia can be divided into obstructive (7%–47%) and non-obstructive categories (53%–93%) [3].

Obstructive azoospermia is a condition characterized by normal levels of spermatogenesis and genital tract obstruction at various levels that prevents the transport of sperm and thus results in the absence of sperm in the ejaculate. Non-obstructive azoospermia (NOA) is defined as a condition in which there is a deficiency of several degrees in the process of spermatogenesis [4]. Non-obstructive azoospermia refers to minimal or no production of fully developed spermatozoa in the testicles. For patients with testicular failure, there is no corrective approach available. This is the most difficult type of male infertility to treat. In some cases of NOA, spermatogenesis may exist in focal areas of the testicular tissue [5]. Testicular volume, serum follicle stimulating hormone (FSH) levels, serum inhibin B levels, and histopathology from fragments of testicular parenchyma obtained via biopsy have been suggested as parameters that differentiate between patients with a relatively high vs. a relatively low chance of having testicular sperm cells retrieved.

Previous studies have shown that elevated FSH levels have been associated with a low probability of retrieving spermatozoa in men. Patients' characteristics revealed FSH levels of <20 IU/L as a cutoff for successful testicular sperm extraction (TESE). Unfortunately, neither FSH, nor histology pattern, nor any of the above-mentioned parameters was able to reliably predict the chances of retrieving sperm in NOA patients on an individual basis. Indeed, there is an ongoing debate in the literature regarding the features that should be employed as predictors of successful sperm retrieval [6-8]. Therefore, we undertook to explore our data regarding the probability of retrieving sperm by means of TESE using any available parameter that might be linked with successful sperm retrieval, including patients' ages, ethnic origins, hormonal profiles, and, more specifically, their FSH, LH, and testosterone serum levels, clinical manifestations such as testis size at clinical examination, and histopathological diagnoses from samples obtained by

TESE.

2. Methods

The present retrospective study included patients diagnosed with NOA and referred to the IVF Unit at Soroka University Medical Center, a tertiary university-based medical center. All the patients underwent TESE and the recorded data was collected and studied retrospectively. The study was approved by the local institutional review committee. NOA diagnosis was based on semen sample evaluation performed on at least four different occasions using 600 g centrifugation and screening at 400 x magnification in accordance with the WHO criteria [9].The absence of sperm in investigated semen samples was followed by physical examination, hormonal panel, and genetic evaluation. A clinical history was recorded, including history of undescended testis, mumps orchitis, previous genitourinary infection, radiotherapy, chemotherapy, or surgical procedures. Clinical examination included secondary sexual characteristics, testicular size, varicocele, hydrocele, and hernia. FSH, luteinizing hormone (LH), and testosterone (T) concentrations were measured and karyotype and Y-chromosomal microdeletion analyses were conducted for all patients. Patients with NOA due to chemotherapy and/or radiotherapy and patients undergoing hormonal treatment, as well as those with obstructive azoospermia, were excluded.

2.1 TESE in brief

Under general anesthesia and with stabilization of the testicle, a small incision was made in the mid-portion of the testicle, through the skin, tunica vaginalis, and tunica albuginea. Tiny multiple samples of testicular tissue were collected using scissors and placed in a Petri dish containing follicle flush medium (William A. Cook Australia, Brisbane Technology Park, Brisbane, Australia). The fluid content of the specimen was evaluated under a microscope in order to find sperm cells. The presence of sperm was reevaluated following centrifugation of the fluid. In order to release the sperms from the seminiferous tubules, the tissue was mechanically dispersed by mincing between two sterile slides, and the presence of sperm was checked under an inverted microscope at × 400 magnification. Thereafter, the tissue specimen was aspirated using 17-gauge and 18-gauge syringes (Becton Dickinson, India) and suspended. The fluids of the suspension were further centrifuged together with the fluid content of the dishes at 755g for 15 minutes, and the pellet was further examined under a microscope in order to identify sperm cells. Once spermatozoa were found, the surgical procedure was terminated. If no spermatozoa were observed, several specimens of tissue were extracted from the contralateral testicle using the same technique and processed the same way. The surgical procedure was always performed by the same surgeon. A fragment of testicular parenchyma was processed for histology and examined by the same laboratory team [10, 11].

2.2 Histology

The sample fragments were washed in buffered medium and delivered to the histology laboratory for evaluation of the tissue. The examinations were performed by the same team of pathologists and the samples were classified according to histopathological pattern: normal spermatogenesis, hypospermatogenesis (reduction in the number of normal spermatogenetic cells), maturation arrest (absence of later stages of spermatogenesis), and Sertoli-cell-only syndrome (absence of germ cells).

2.3 Statistical analysis

Continuous variables with normal distribution are presented as mean and standard deviation. Ordinary variables or continuous variables with non-normal distribution are presented as medians with an interquartile range (IQR). Categorical variables are presented as counts and percentages of the total. The

preferred method of analysis for continuous variables was parametric and used a Student's t-test. Non-parametric procedures were used if parametric assumptions could not be satisfied even after data transformation attempts, and included the Mann-Whitney test. Parametric model assumptions were assessed using Normal probability plot or Shapiro-Wilk test for verification of normality and Levene's test for verification of homogeneity of variances. Categorical variables were tested using Pearson's $\chi 2$ test for contingency tables or Fisher's exact test, as appropriate. Multivariate logistic regression was used to assess the adjusted effects on the probability for successful TESE after variable interactions were ruled out. All statistical tests and/or confidence intervals, as appropriate, were performed at α=0.05 (2-sided). All p-values reported were rounded to two decimal places. The data was analyzed using IBM SPSS Statistics software.

3. Results

The present study included 119 NOA patients with a mean age of 32.99 ± 7.83 years who underwent TESE by the same operator. Seventy-eight (78) (65.54%) of the extractions were successful and 41 (34.45%) failed to retrieve sperm (Table 1). Interestingly, the age of patients with successful TESE was significantly higher than the age of those whose sperm retrieval failed, i.e., 34.32 ± 6.67 and 29.37 ± 9.59 years, respectively ($p<0.02$). Analyzing our data according to patients' ethnic origin demonstrated a significantly higher sperm retrieval rate among 57 patients of Jewish origin (mean age 35.3 ± 6.67 years) compared with 62 Bedouin patients (mean age 30.77 ± 8.28 years)—44 (77.19%) versus 34 (54.8%), respectively ($p<0.01$), emphasizing a significantly younger patient population among the Bedouin patients with a much lower sperm retrieval rate. The 119 histological samples (Table 2) from the tissue fragments collected during TESE were examined by an experienced pathologist. In 41 (34.4%) patients, diagnosis of Sertoli-cell-only syndrome was established, but interestingly, the IVF laboratory was able to identify sperm in 25 of these patients (61%). Similarly, maturation arrest was diagnosed by the pathologist in 38 (31.9%) cases, while examination of all of the tissue retrieved during TESE yielded 23 (60.5%) successful mature sperm retrievals. Furthermore, hypospermatogenesis was established by the pathologist in 31 (26%) patients, while sperm retrieval was successful in 22 (71%) patients. Trends toward testicular volume below 10ml and elevated FSH levels (>12 mIU\ml) were observed among patients with unsuccessful sperm retrieval.

As Table 3 shows, FSH levels >12mIU\ml were found in 76 (63.8%) patients, with a mean value of 25.84± 12.63. LH levels were not correlated with high FSH levels ($p<0.001$) or low testosterone levels. Comparison of histological diagnosis of TESE fragments with patients' FSH levels demonstrated a significant association between elevated FSH levels in 35 of 41 (85.4%) cases with Sertoli-cell-only syndrome. A similar trend, but lower prevalence of elevated FSH, was found among patient diagnosed with maturation arrest (65.8%), while the lowest association obtained was related to hypospermatogenesis (48.4%). Genetic abnormalities were observed in 14 (11.7%) patients. Eight of them had Klinefelter syndrome, and all of them presented with elevated FSH levels, ($p<0.05$). The testicular volume on clinical examination was not correlated with the histology of the samples, FSH, or testosterone levels. A multivariate logistic regression analysis was performed with the aim of revealing any variables independently correlated with successful sperm retrieval by TESE (Table 4). Among all the variables in this study, only the age of the patients (older) and, marginally, ethnic origin correlated independently with the probability of successful sperm retrieval in NOA patients.

	Patients (age y. ± SD)	Jewish (age y. ± SD)	Bedouin (age y. ± SD)	p-value
N	119 (32.99 ± 7.83)	57 (35.3 ± 6.67)	62 (30.77 ± 8.28)	
Successful TESE	78 (34.32 ± 6.67)	44 (35.52 ± 6.90)	34 (32.88 ± 6.07)	0.08
Unsuccessful TESE	41 (29.37 ± 9.59)	13 (33.31 ± 5.36)	28 (28.46 ± 9.43)	0.1
p-value	0.02	0.01	0.01	
Success rate (%)	65.57	77.19	54.88	<0.01

Table 1: Ethnic and age parameters in relation to the probability of retrieving sperm by means of TESE.

Variables		Entire group (n=119)	Successful TESE (n=78)	Unsuccessful TESE (n=41)	p-value
BMI, mean (SD)		27.62 (5.83)	27.11 (4.26)	28.76 (8.38)	0.4
Sperm Retrievals (TESE)		119	78 (65.54%)	41 (34.45%)	
Histopathology No (%)	Sertoli-cell-only syndrome	41 (34.4%)	25 (61%)	16 (39%)	0.45
	Maturation arrest	38 (31.9%)	23 (60.5%)	15 (39.5%)	0.43
	Hypos-permatogenesis	31 (26%)	22 (71%)	9 (29%)	0.46
	Normal	9 (0.07%)	8 (88.9%)	1 (2.4%)	0.16
Right testicular volume (ml), No. (%)	<9.9	21 (17%)	12 (57%)	9 (42.8%)	0.55
	>10	97 (82.2%)	65 (67%)	32 (32.9%)	
Left testicular volu-me (ml), No. (%)	<9.9	24 (20.4%)	14 (58.3%)	10 (41.6%)	0.37
	>10	94 (79.6%)	64 (68%)	30 (31.9%)	
FSH values > 12 mIU/ml, n (%)		76 (63.9%)	26 (34.2%)	50 (65.7%)	0.94

Table 2: Patients' physical and histopathologic characteristics related to successful TESE.

Variables		Entire group (n=119)	Normal FSH -43	High FSH (n=76)	p-value
FSH (mIU/ml), mean ± SD		18.99 ± 13.74	6.57 ± 25.84	25.84 ± 12.63	n/a
LH (mIU/ml), mean ± SD)		8.21 ± 5.29	5.72 ± 4	9.62 ± 5.44	<0.001
Testosterone (ng/dl), mean ± SD		6.38 ± 5.62	7.62 ± 6.47	5.69 ± 5	0.1
Histopathology No (%)	Sertoli-cell-only syndrome	41 (34.5%)	6 (14.6%)	35 (85.4%)	<0.001
	Maturation arrest	38 (31.9%)	13 (34.2%)	25 (65.8%)	0.77
	Hypospermatogenesis	31 (26.1%)	16 (51.6%)	15 (48.4%)	0.04
	Normal	9 (7.6%)	8 (88.9%)	1 (11.1%)	0.001
Genetic abnormalit-ies, No (%)	Y-Microdeletion A	1 (0.8%)	1	-	n/a
	Y-Microdeletion C	3 (2.5%)	1	2	
	Y-Microdeletion D	2 (1.7%)	-	2	
	Klinefelter	8 (6.7%)	-	8	0.05

Testicular volume above 10ml [a] No (%)	Right	93 (79.5%)	37 (39.8%)	56 (60.2%)	0.08
	Left	91 (77.1%)	36 (39.6%)	55 (60.4%)	0.1
FSH: high (> 12 mIU/ml) LH: high (>12mIU/ml) Testosterone: low (<2ng/ml)					

Table 3: Histopathological, genetic, and physical parameters related to the FSH levels in patients undergoing TESE.

Variable	Odds Ratio	95% CI for Odds Ratio	p-value
Age	1.08	1.005-1.149	0.04
Ethnicity	0.42	0.16-1.12	0.08
Testicular volume above 10 ml	1.46	0.5-4.3	0.49
High FSH	0.58	0.18-1.87	0.34
Low Testosterone	0.84	0.15-4.57	0.84

Table 4: Multivariate logistic regression analysis to predict successful TESE.

4. Discussion

While great advances have taken place in the field of IVF in recent decades, the ability to provide NOA patients with accurate predictions regarding their possible TESE success rates remains suboptimal. Our need as clinicians for better predictive tools for this patient population extends beyond shared scientific interest, since the consequences of failure to retrieve sperm may be severe in emotional and financial terms and may also affect patients' health. Therefore, the need to obtain more reliable parameters for the prediction of TESE outcomes in NOA patients remains of paramount importance. Several parameters were taken into consideration as predictors of successful testicular sperm retrieval. The role of FSH in spermatogenesis is already well documented. FSH acts on Sertoli cells, which are the only cells in the male reproductive system with FSH receptors [12]. FSH levels discriminate well between obstructive and NOA states, with the former demonstrating normal levels and the latter high levels [13]. Nonetheless, FSH values cannot discriminate between the subtypes of NOA due to the wide overlapping of FSH values even in normozoospermia. Nevertheless, studies have shown that FSH levels inversely correlated with sperm retrieval in NOA [14].

In fact, the present study showed elevated FSH in 85.4% of patients with histopathology of Sertoli-cell-only syndrome, correlated with a sperm extraction rate of 61%, which was found to be similar to the rate in cases of maturation arrest, but lower than the rate found in patients diagnosed hypospermatogenesis (71%). Our patients underwent conventional TESE, with a mean sperm retrieval rate of 65.54%. Most of them had unexplained hypergonadotropic NOA, only 6.7% were diagnosed with Klinefelter syndrome, and 6 had Y-microdeletions. We demonstrated a significant and positive correlation between increased patient age and successful TESE, i.e., a mean of 34.3 years for the successful sperm retrievals and 29.4 years for the failed ones. The findings related to patient age are in line with those of a study by Gnesi et al. [15] that examined 486 patients undergoing TESE, but contradict results from a meta-analysis published by Corona et al. on patients

with Klinefelter syndrome [16]. Indeed, our data among patients diagnosed with Sertoli-cell-only syndrome by means of a random biopsy of testicular parenchyma using conventional-TESE revealed that 61% achieved successful sperm retrieval, regardless of their extremely high FSH levels, emphasizing the inability of these parameters to accurately predict the success of sperm extraction.

Interestingly, TESE results in terms of successful sperm retrieval according to the ethnic origins of patients have demonstrated a significantly higher success rate among patients of Jewish origin—77.19% as opposed to 54.88% among patients of Bedouin origin. In addition, the age of the patients of Jewish origin was significantly higher (35.3 ± 6.67 years) than that of the Bedouin patients (30.77 ± 8.28 years). The age differences are most probably related to the fact that patients of Bedouin origin traditionally marry and begin planning families at a younger age than their Jewish counterparts, so they come to the attention of infertility specialists at relatively younger ages. Several studies have shown racial and sociodemographic differences in semen parameters [17, 18] in the range of the upper or lower limits of normozoospermia or oligozoospermia and even in the prevalence of azoospermia. A recent study conducted by our group revealed a genetically hereditary mutation in the TDRD9 gene that is associated with NOA in a certain Bedouin tribe [19] and more studies are under way to identify similar genetic abnormalities correlated with male infertility [20] as well as congenital cardiovascular abnormalities [21] and many other anomalies [22].

Based on the present study, we may conclude that we could not identify a single parameter able to predict successful TESE. Clearly, the ethnic origin of our patients has proved to be an important parameter. It may be related to the genetic background of the different populations, especially given that consanguineous marriage is a common practice in certain communities. To the best of our knowledge, this is the first study to reveal an impact on the probability of retrieving sperm in NOA patients based on ethnic origin in a vast population, not based on a specific gene abnormality, but most probably indicating underlying genetic derangements related to sperm production. Therefore, we suggest considering data regarding ethnic origin in the prediction of TESE success in addition to the traditionally investigated parameters. Obviously, continuous genetic investigation of consanguineous families and tribes with male infertility could contribute to improved prediction of sperm retrieval in their members. Additional studies that include ethnic origin as a parameters related to NOA should be undertaken to corroborate our findings.

Statement of Ethics

The authors have no ethical conflicts to disclose

Funding Sources

No funding sources to declare

Author Contributions

A.Z. and N.K conceived the original idea and collected the data, Y.L performed data analysis, El. Le. and Et. Le. And Na.St. wrote the manuscript with support from I.H. and A.B., Ei. Lu. supervised the project.

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