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Predicting Factors for the Outcome of Testicular Sperm Extraction in Men with Azoospermia

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ABSTRACT

Backgrounds: Infertility is a common disease that impacts about 20% of couples, with male infertility contributing to roughly half of these cases. Testicular Sperm Extraction (TESE) is the main treatment for male infertility with Non-Obstructive Azoospermia (NOA). Appropriate counseling regarding sperm extraction approaches for infertile couples is crucial to decide further treatments and to expect achievable outcomes.

Aims: Our purpose is to identify potential clinical predictor factors for TESE outcomes.

Material and Methods: The study was conducted prospectively in 2019-2022. We recruited 48 male patients with diagnosis of azoospermia in the Infertility and Reproductive Center, National Center for Maternal and Child health in Mongolia.

Results: Mean age of males were 35.0 ± 5.03 with range of 22-45. Spermatozoa were successfully obtained from 32 (66.7%) men. There was statistically significant higher level of Follicle-Stimulating Hormone (FSH) of 19.7 ± 12.3 (p=0.0007) in the failed sperm retrieval group comparing successful sperm retrieval group. Researchers used receiver operating characteristics curve to determine which FSH threshold level resulted successful retrieval of spermatozoa. Of 58.3% patients with FSH less than 12.4U/L or normal had spermatozoa and 8.3% patients had without spermatozoa. The mean level of FSH in the cases without spermatozoa was 19.7 ± 12.3 IU/L. It was significantly higher than the cases with spermatozoa (p=0.007). FSH level of 12.4 mIU/mL is 90% sensitive to predict 90.0% of spermatozoa retrieval. We carried out 29 embryo transfers which was resulted 31.0% pregnancy.

Conclusion: It is possible to assess the outcome of TESE procedures based on the level of FSH in the serum.

Keywords: Azoospermia; Sperm retrieval; Testicular sperm extraction

INTRODUCTION

Infertility is a common disease that impacts about 20% of couples, with male infertility contributing to roughly half of these cases [1]. Azoospermia represents a severe manifestation of male infertility, characterized by the total absence of spermatozoa in the semen [2]. It impacts 10% to 15% of men experiencing infertility and is further categorized into Obstructive Azoospermia (OA) and Non-Obstructive Azoospermia (NOA) [3]. OA is approximately 40% of cases, results most commonly from obstruction in the ductal system. The clinical management of obstructive azoospermia depends on its cause [4]. NOA accounts for around 60% of cases and typically stems from spermatogenetic dysfunction, which can manifest as primary testicular failure, secondary testicular failure, or incomplete testicular failure. Prior to microsurgical testicular sperm retrieval techniques and IVF/ICSI, donor insemination was the only option available to men with NOA [4,5].

Presently, advancements in medical technology offer couples the opportunity to conceive biological offspring through procedures like Testicular Sperm Aspiration (TESA), Conventional Testicular Sperm Extraction (cTESE) or microdissection TESE (mTESE) [6,7]. Conventional TESE has lower costs and high reproducibility, therefore still support this procedure associated with Intracytoplasmic Sperm Injection (ICSI) as the first line treatment in NOA [8]. But these treatments have the possibility of failure and predicting the sperm retrieval rate before surgery is important [9]. Appropriate counseling regarding sperm extraction approaches for infertile couples is crucial to decide further treatments and to expect achievable outcomes.

Regarding to predicting of TESE outcomes, some parameters, including endocrine markers and predictive models were using in clinical practice. Lower Luteinizing Hormone (LH) and Follicular Stimulating Hormone (FSH) values were significantly associated with positive Sperm RETRIEVAL RATE (SSR) with Testicular Sperm Aspiration (TESA) [10]. In the prospective cohort study, the successful SRR with c-TESE was 47.6% and suggested that serum FSH and LH levels, overall histopathology diagnosis and mean Johnsen score represented the most accurate predictors of successful SRR [8]. Moreover, studies reported that serum FSH level can predict the SRR of NOA and addressed the cut-off values for FSH to guide the clinicians while selecting the suitable surgery approach for NOA [11,12]. Using endocrine markers is minimal invasive and inexpensive, so that it is suitable markers in care for infertile couples.

The predictors of surgical sperm retrieval in patients with non-obstructive azoospermia have been widely studied, but there is no consistent clinical or laboratory factor available to counsel patients with NOA regarding their prospects of TESE success [9,10]. The National Center for Maternal and Child Health (NCMCH) offers national infertility care services. There is need for assess the predictor variables that can predict the presence of sperm. Our purpose is to identify potential clinical predictor variables for TESE outcomes.

MATERIALS AND METHODS

Setting and participants

This prospective study included 48 men who were diagnosed with azoospermia and underwent open TESE for the first time between 2019 and 2022 at the Infertility and Reproductive Center of the Obstetrics and Gynecology Hospital, National Center for Maternal and Child health. The study recruited men aged 18-45 who had azoospermia confirmed by two semen analysis in accordance with 5th WHO guidelines. Azoospermia was considered in the absence of spermatozoa in semen. Inclusion criteria included patients with a diagnosis of male infertility, who agreed to be participated in the study and reproductive age. Men diagnosed with comorbidities affecting TESE outcomes were excluded.

Data collection technique

Study data were collected through questionnaires, laboratory, and ultrasound. Informed consent was obtained from study participants, and ultrasound and laboratory parameters potentially predictive of TESE outcome were identified before treatment.

Measured predictive factors: Previous studies have measured the following key predictors. Participant's age and Body Mass Index (BMI) were measured according to guidelines. The following hormone variables were measured. Serum Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and prolactin and testosterone analysis were performed on fully automated analyzer Hitachi Cobas e-411. Testicular parameters were measured by ultrasound. Testicular size was measured by length, width and thickness using linear array probe the Samsung G-60 ultrasound machine: the volume was calculated by the formula.

v = length × width × thicness × 0.52

TESE technique and measured outcomes: Testicular Sperm Extraction (TESE) was performed under spinal anesthesia. Testicular tissue was transported to the embryology laboratory in pre-prepared incubator plates containing numbered HTF solutions. The tissues were cut into sections under stereo microscope and viewed with a Nikon phase contrast microscope to identify and count sperm cells. If no sperm was found on all specimens, the patient was informed and the tissue was discarded. Study participants who testicular spermatozoa were retrieved; Intracytoplasmic Sperm Injection (ICSI) carried out. ICSI is procedure of injecting live sperm into an oocyte in a laboratory environment or in vitro. This procedure leads to fertilize of oocyte in order to create an embryo. Embryo transfer is the final procedure of the *in vitro* fertilization process that involves transfer of one or more embryos into the uterine cavity typically by using a catheter inserted through the uterine cervix. We assumed that inter-individual and inter-procedure differences were low because ICSI was performed by a single experienced embryologist according to protocol. All retrieved sperm was frozen and stored. TESE outcomes were measured by SRR and pregnancy.

Statistical analysis

Data were collected into MS Excel and statistical analysis performed using R 4.3.1 software. The distribution of quantitative variables was estimated as mean and standard deviation. Differences in the mean of hormone parameters between TESE successful and unsuccessful groups were measured by independent two sample T-test. A independent two-sample T test was also used to compare the difference in the mean of hormone parameters with respect to testis size. Multivariate linear regression analysis was used to estimate factors influencing TESE outcomes. The sensitivity and specificity of FSH and LH were calculated using the Youden's index.

Ethical considerations

Research ethics approval was obtained from Ethical committee of National Center for Maternal and Child Health. Consent form was obtained from the all participants.

RESULTS

General characteristics

The mean age of patients underwent TESE was 35.0 ± 3.86, with the youngest age of 23 and the oldest age of 45. As for the distribution of paternal age group, 33.3% (n=16) were 30 years to 35 years old, and 18.8% (n=9) were over 40-year-old. Body weight ranged from 54 kg to 119 kg, with an average of 80.58 kg, and height ranged from 152 to 186 cm with an average of 170.5 cm (Table 1).

Table 1: Main characteristics of the participants.

Main characteristics		n	%
Paternal age (mean ± SD)		35.0 ± 3.86	
Maternal age (mean ± SD)		33.5 ± 4.1	
Age group	23-29.9	8	16.7
	30-34.9	16	33.3
	35-39.9	15	31.3
	40-45	9	18.8
Paternal BMI	<18	1	2.1
	18.5-24.9	15	31.3
	25-29.9	19	39.6
	>30	13	27.1
Married or with partner		48	100

Out of patients underwent TESE, 89.5% (n=43) had primary infertility, 10.4% (n=5) had secondary infertility, and 62.5% (n=30) had infertility for 2 years to 10 years (Table 2).

Table 2: Reproductive characteristics of participants.

Infertility variables		Number	Percentage (%)
Type of infertility	Primary	43	89.5
	Secondary	5	10.4
Infertility period	<2 years	6	12.5
	2-10 years	30	62.5
	>10 years	12	25

Patients had highest FSH concentration of 53.8 IU/L and lowest of 1.43 IU/L, and for LH concentration with highest of 23.0 IU/L and lowest of 2.1 IU/L (Table 3).

Table 3: Main measurements of participants.

Variables	Mean ± SD	Maximum	Minimum
FSH (IU/L)	8.27 ± 9.9	53.8	1.43

LH (IU/L)	6.6 ± 4.02	23	2.1
Testosterone (ng/ml)	3.97 ± 1.64	8.05	1.8
Prolactin (ng/ml)	15.5 ± 7.8	7.5	49
Testicle size (cm3)	22.3 ± 12.0	3.09	56.6

Testicular spermatozoa retrieved in 66.7% (n=32) patients. In patients with FSH level less than 12.4U/L, 58.3% (n=28) patients with spermatozoa and 8.3% (n=4) patients without spermatozoa. Spermatozoa cells were detected in 62.5% (n=30) patients with testosterone level of more than 2.8 ng/ml, while spermatozoa not detected in 27.1% (n=13) patients with testosterone with less than 2.8 ng/ml. Spermatozoa retrieved in 12.5% (n=6) patients with prolactin levels of more than 21.4 ng/ml (Table 4).

Table 4: Comparison of the outcome of TESE with the type of infertility and level of serum hormones.

		Spermatozoa (+)(n=32)		Spermatozoa (-) (n=16)	
		n	%	n	%
Infertility period	<2 years	6	12.5	0	5.6
	2-10 years	20	41.7	10	20.8
	>10 years	6	12.5	6	12.5
FSH	Less than 12.4 IU/L	28	58.3	4	8.3
	More than 12.4U/L	4	8.3	12	25
Testosterone	>2.8ng/ml	30	62.5	13	27.1
	<2.8ng/ml	2	4.1	3	6.3
Prolactin	<21.4 ng/ml	26	54.2	14	29.2
	>21.4 ng/ml	6	12.5	2	4.2

Patients underwent TESE were divided into 2 groups: with spermatozoa and without spermatozoa. Comparing some biometric parameters between groups, the mean of FSH level in the cases without spermatozoa was 19.7 IU/L ± 12.3 IU/L. It was significantly higher than the cases with spermatozoa (p=0.007). There were no difference between the two groups in age, body weight and other hormone parameters (Table 5).

Table 5: Comparison of the outcome of TESE.

	Spermatozoa (+) (n=32)	Spermatozoa (-) (n=16)	T test p-value
	Mean ± SD	Mean ± SD	
Age	35.5 ± 5.0	34.0 ± 5.1	0.341
Period of infertility	6.3 ± 4.44	7.8 ± 4.21	0.252
Testicle size (cm3)	26.11 ± 11.3	13.5 ± 7.9	0.0001
BMI	27.2 ± 3.2	28.2 ± 6.1	0.51
FSH (IU/L)	6.77 ± 4.4	19.7 ± 12.3	0.0007*
LH (IU/L)	6.9 ± 2.483	9.88 ± 5.17	0.09
Testosterone (ng/ml)	4.53 ± 1.59	4.25 ± 1.8	0.6
Prolactin (ng/ml)	15.45 ± 6.58	15.7 ± 10.4	0.92

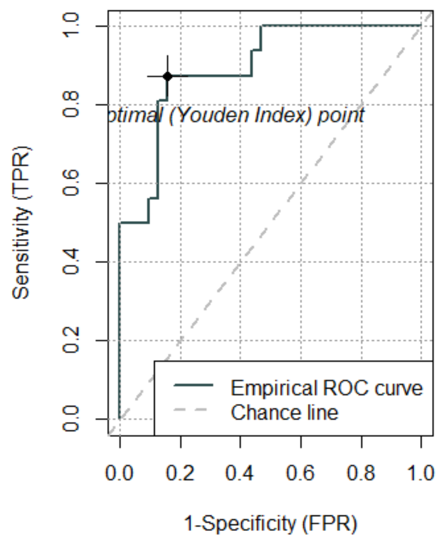

Figure 1: FSH concentration and sensitivity of testicular spermatogenesis.

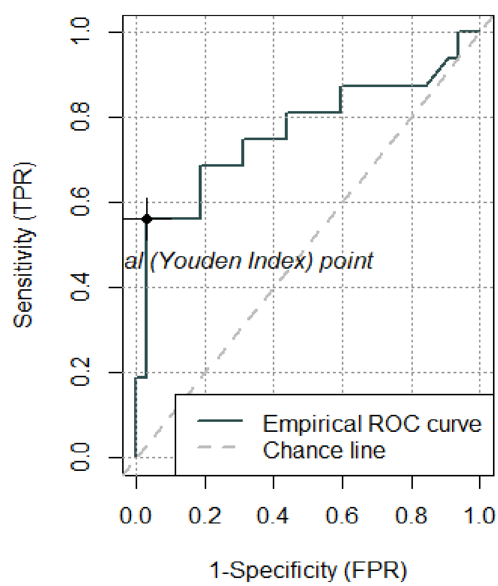

Figure 2: LH concentration and sensitivity of testicular spermatogenesis.

The researchers determine the relationship between FSH and testicular spermatogenesis using the ROC curve. The sensitivity of FSH for spermatogenesis was 88.0%, while LH was 56% (Figure 1,2).

The men who participated in the study were divided into 2 groups according to testicle size, the mean levels of FSH and prolactin in men with normal testicle size were 3.75±1.61 IU/L and 11.89 ng/ml ± 3.16 ng/ml, respectively, which was statistically lower than the other group. There was no difference between the two groups in age, body weight, and other hormone parameters (Table 6).

Table 6: Comparison of testicular size between normal and small testicle.

	Normal testicle (> TTV 32 cc, n=8)	Small testicle (< TTV 32 cc, n=40)	T test p-value
	Mean ± SD	Mean ± SD	
Age	37.6 ± 6.1	34.8 ± 4.7	0.204
Period of infertility	5.1 ± 2.74	7.1 ± 4.59	0.11
BMI	27.8 ± 1.74	27.5 ± 4.74	0.75
FSH (IU/L)	3.75 ± 1.61	12.6 ± 10.3	0.000*
LH (IU/L)	5.8 ± 2.36	7.5 ± 4.24	0.134
Testosterone (ng/ml)	5.2 ± 1.51	4.29 ± 1.6	0.172
Prolactin (ng/ml)	11.89 ± 3.16	16.27 ± 8.25	0.010*

In a multivariate regression analysis, age, infertility period and testosterone level were not associated with factors that may influence SRR. The FSH concentration was statistically significant with a p-value of 0.005 (Table 7).

Table 7: Multivariate regression analysis of factors influencing successful Sperm Retrieval(SRR).

	Regression coefficient	T test p-value
Infertility period	0.57	0.56
BMI	1.5	0.12
FSH	2.96	0.005
Testosterone	0.93	0.35

All participants with testicular spermatozoa underwent ICSI, and the total of 197 oocytes were retrieved from 29 women. Number of fertilized oocytes were 150 (76.1%). Mean of 2.2 embryos were implanted. 9 (31.0%) women became pregnant. Currently, 1 woman is pregnant, 1 woman had preterm birth and 7 women had normal live birth (Table 8).

Table 8: Outcome of TESE and ICSI.

	n
Maternal age (mean, range)	33.58 (23-43)
Number of retrieved oocytes (mean, range)	6.79 (2-23)
Number of embryos implanted (mean, range)	2.2 (1-3)
No of retrieved oocytes	197
No of fertilized oocytes	150
No of pregnancy	9
No of live birth	7
No of preterm birth	1
No of currently pregnant	1

Table 9 shows comparison of paternal characteristics between pregnant and non-pregnant women.

Table 9: Comparison of some indicators of men in pregnant and non-pregnant female partner's groups.

		Pregnant (n=9)		Non-pregnant (n=20)		Chi-quadrant P value
		n	%	n	%	
Pregnancy in result of TESE-ICSI		9	31	20	69.1	
Type of infertility	Primary	7	24.1	19	65.5	0.018
	Secondary	2	6.9	1	3.4	
Period of infertility (years)	<2	-	-	3	10.3	0.31

	10-Feb	7	24.1	13	44.8	
	>10	2	6.9	4	13.8	
FSH	Less than 12.4U/L	6	20.7	17	58.6	0.021
	More than 12.4U/L	3	10.3	3	10.3	
Testosterone	>2.8ng/ml	8	28.6	16	57.1	0.102
	<2.8ng/ml	1	3.8	3	10.7	
Prolactin	<21.4 ng/ml	2	20	5	50	0.25
	>21.4ng/ml	1	1	2	20	

6. Discussion

In this study, the mean age of participants was 35.0±3.8, which is similar to the age of 32.6 ± 0.48 in the study of Chehrazi M [13]. As well as, Yi ru et al reported there were 76.0% (n=140) patients aged 30-39, while in our study there were 61.1% (n=22) people aged 30-39 [14]. These results indicate that patients, underwent sperm extraction procedure from testicle and IVF treatment due to the absence of sperm in the semen, are usually men of reproductive age between 30 and 39 years of age. We divided the participants into two groups by type of fertility: primary and secondary infertility. In a study of Vaidyanathan Gowri (2010), there were 67(68.3%) patients with primary infertility and 31 (31.6%) patients with secondary infertility, which is different from our study [15].

In our study, we evaluated the model to predict the outcome of TESE in patients with azoospermia by hormonal parameters, age, testicle size and BMI. Previous studies have reported predictability with these variables [6,16,17]. Recent studies concerning the prediction of TESA success in patients with azoospermia has primarily centered on factors such as age, BMI, testicular volume, and serum hormone levels [18]. Study reported, FSH level, age and testicular volume were included in the prediction model for sperm retrieval failure risk [18].

There are few studies on the relationship between male age and fertility outcome. The evidence regarding its negative impact on fertility has been inconsistent [19-22]. One observational study, it was found that fertility declined with increasing age for both men and women, particularly among men with non-obstructive azoospermia. Researchers recommended early consultation with a doctor, which appears to have a beneficial impact on fertility outcomes [23]. But several studies have found no relationship between male age and the outcome of mTESE procedures. Surprisingly, the overall sperm retrieval rate was highest among men aged 40 years or older, suggesting that there is no age limit for the success of micro-TESE [24-27]. Our result supported these outcomes, with no significant difference in mean age observed between the groups where patients with spermatozoa and without spermatozoa.

The researchers are evaluating the correlation between the body mass index and the outcomes of TESE procedures. However, the patient's BMI was unable to predict a positive sperm retrieval outcome [27,28]. Nevertheless, a negative correlation was identified between serum testosterone levels and BMI, suggesting a potential impact on fertility [28]. Our study result is consistent with these outcomes.

Studies suggested that LH, prolactin, and total testosterone do not serve as predictors of the sperm retrieval rate in patients undergoing TESA with non-obstructive azoospermia [16,29]. Testosterone levels could not be diagnostic value for non-obstructive azoospermia is similar to results of our study.

Conversely, the predictive relevance of FSH and estradiol remains a topic of debate in other studies [29,30]. Turuncl et al. [31] suggested that FSH could not be used as a factor to predict the success of testicular sperm retrieval in patients with NOA [32]. Kızılay F et al. [33] evaluate to the sperm retrieval and factors affecting these rates in men who underwent repeat mTESEs [33]. This study results confirm testicular volume, histology, karyotype, and Y-chromosome microdeletion were predicting factors for successful sperm retrieval. Gnessi L also developed a model for predicting outcomes in which younger men and lower FSH levels are important [34]. In a 2010 study of 206 men at the Infertility center of National Taiwan University Hospital, comparing mean FSH levels with sperm retrieval results, when FSH level was above the level of 19.4 mIU/mL, there were no successful sperm retrievals. Also in this study, the failed sperm retrieval group had mean FSH level of 28.03 mIU/mL ± 14.56 mIU/mL, while the successful group had 7.94 mIU/mL ± 4.95 mIU/mL [35]. In our study, it was 19.7 mIU/mL ± 12.3 mIU/mL in unsuccessful sperm retrieval cases and 6.77 mIU/mL ± 4.4 mIU/mL in successful sperm retrieval cases, which are similar results. Study with 944 infertile patients conducted in Indonesia (2016) reported that FSH level was 8.53 mIU/mL ± 8.43 mIU/mL for obstructive azoospermia and 20.12 mIU/mL ± 11.89 mIU/mL for non-obstructive azoospermia ($p<0.001$) and FSH value above 10.36 mIU/mL had sensitivity 82.1% and specificity 79.5% for predicting non-obstructive azoospermia [36].

The limitation of our study is that the sample size is relatively small. We recruited all patients with azoospermia confirmed by semen analysis. We did not determine patients with genetic abnormality such as abnormal karyotype or presence of Y chromosome microdeletions. Also, in this study sperm retrieval in cases where microscope is not available, studies revealed that mTESE was 1.5 times more likely to result in successful sperm retrieval [37]. The strength of our investigation is the prospective design of the study, even though small number of patients was participated. Our study suggests that it is possible to predict TESE outcomes based on the serum FSH level. Additionally, this study has provided definitive cut-off values for FSH measurement.

7. Conclusion

When the serum FSH level is 12.4 IU/mL or less, the success rate of sperm extraction is 90%. Testosterone and serum LH levels were poor predictors of testicular spermatogenesis.

8. Authors' Contributions

All authors have contributed equally to the drafting of the manuscript. All authors read and approved the final version of the manuscript.

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