Journal of Clinical Obstetrics, Gynecology & Infertility

Research Article
Published: 23 Mar, 2024

Outcome of Testicular Sperm Extraction in Men with Azoospermia

Dagvadorj B, Enkhtaivan O, Myagmartseren P and Enkhmaa D*

National Center for Maternal and Child Health, Mongolia

Abstract

Background: 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). Our purpose is to identify potential clinical predictor variables for TESE outcomes.

Materials 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 was 35.0 ± 5.03 with range of 22 to 45. Spermatozoa were successfully obtained from 32 (66.7%) men. There was statistically significant higher level of Follicle-stimulating hormone of 19.7 ± 12.3 (p-value 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. 58.3% (n=28) patients with FSH less than 12.4 U/L or normal had spermatozoa and 8.3% (n=4) 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 9 (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; FSH; Testicular sperm extraction

OPEN ACCESS

***Correspondence:**
Davaasambuu Enkhmaa, National Center for Maternal and Child Health, Khuvisgalchdiin Street, Bayangol District, Ulaanbaatar 16060, Mongolia;

Received Date: *22 Feb 2024*
Accepted Date: *18 Mar 2024*
Published Date: *23 Mar 2024*

Citation:
Dagvadorj B, Enkhtaivan O, Myagmartseren P, Enkhmaa D. Outcome of Testicular Sperm Extraction in Men with Azoospermia. J Clin Obstet Gynecol Infertil. 2024; 8(1): 1064.

Introduction

Infertility is a common disease that impacts about 20% of couples, with male infertility contributing to roughly half of these cases [1,2]. Azoospermia is severe form of male infertility, defined as complete absence of spermatozoa in the semen. Azoospermia affects about 10% to 15% of all males with infertility and classified into Obstructive Azoospermia (OA) and Non-Obstructive Azoospermia (NOA) [3,4].

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 [5]. Esteves et al. reported a cumulative Sperm Retrieval Success Rate (SRR) in men with OA was 97.9% using percutaneous epididymal sperm aspiration, in association or not with Testicular Sperm Aspiration (TESA) regardless of the cause of obstruction [6].

NOA, the etiology affecting approximately 60% of cases, results from spermatogenetic dysfunction either 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 [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) [7,8]. 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 [9].

Since the ability to predict such an outcome would allow the urologist to individuate those patients who are suited for TESE, several prediction models have been developed to date, however their resulting prediction accuracy was never strong enough to translate their results to the clinical practice [10]. Consequently, there is consistent no clinical or laboratory factor available to counsel patients with NOA regarding their prospects of TESE success [11-13]. 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 [9]. 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 [14,15].

The National Center for Maternal and Child Health (NCMCH) offers national infertility care services. There is need for assess the predictive cut-value of markers of serum hormones on SSR and influencing factors of TESE treatment. Our purpose is to identify potential clinical predictor variables for TESE outcomes.

Materials and Methods

Study design/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 23 to 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.

Predictor factors and measurements

Ultrasound factors: 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 length × width × thickness × 0.52.

Hormonal factors: Serum Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and prolactin and testosterone analysis were performed on fully automated analyzer Hitachi Cobas e-411.

TESE technique and Intracytoplasmic sperm injection

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.

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 samples T-test. An 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 study 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 age group of males, 33.3% (n=16) were 30 to 35 years old, and 18.8% (n=9) were over 40-year-old. Age groups was no statistical difference (p=0.24). Body weight ranged from 54 kg to 119 kg, with an average of 80.58 kg, and height ranged from 152 cm to 186 cm with an average of 170.5 cm. There was a statistically significant difference between BMI groups (p=0.001) (Table 1).

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 to 10 years (Table 2).

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 1: Main characteristics of the participants.

Main characteristics		n	%	P-value
Paternal age (mean ± SD)		35.0 ± 3.86		
Maternal age (mean ± SD)		33.5 ± 4.1		
Age group	23-29.9	8	16.7	0.24
	30-34.9	16	33.3	
	35-39.9	15	31.3	
	40-45	9	18.8	
Male BMI	<18	1	2.1	0.001
	18.5-24.9	15	31.3	
	25-29.9	19	39.6	
	>30	13	27.1	
Married or with partner		48	100	

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

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)	6.627 ± 4.02	23	2.1
Testosterone (ng/ml)	3.9727 ± 1.64	8.05	1.8
Prolactin (ng/ml)	15.527 ± 7.8	7.5	49
Testicle size (cm³)	22.327 ± 12.0	3.09	56.6

Table 4: Comparison of the results 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 12.4 U/L	4	8.3	12	25
Testosterone	>2.8 ng/ml	30	62.5	13	27.1
	<2.8 ng/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

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	26.11 ± 11.3	13.5 ± 7.9	0.0001
BMI	27.2 ± 3.2	28.2 ± 6.1	0.51
FSH	6.77 ± 4.4	19.7 ± 12.3	0.0007*
LH	6.9 ± 2.483	9.88 ± 5.17	0.09
Testosterone	4.53 ± 1.59	4.25 ± 1.8	0.6
Prolactin	15.45 ± 6.58	15.7 ± 10.4	0.92

Testicular spermatozoa retrieved in 66.7% (n=32) of the total patients. In patients with FSH less than 12.4 U/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. Among patients with prolactin levels of more than 21.4 ng/ml, spermatozoa were detected in 12.5% (n=6) of cases (Table 4).

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 ± 12.3 IU/L. It was significantly higher than the cases with spermatozoa (p=0.007). There was no difference between the two groups in age, body weight and other hormone parameters (Table 5).

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. When comparing some biometric parameters, the mean levels of FSH and LH in men with normal testicle size were 3.75 ± 1.61 IU/L and 5.8 ± 2.36 IU/L, 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).

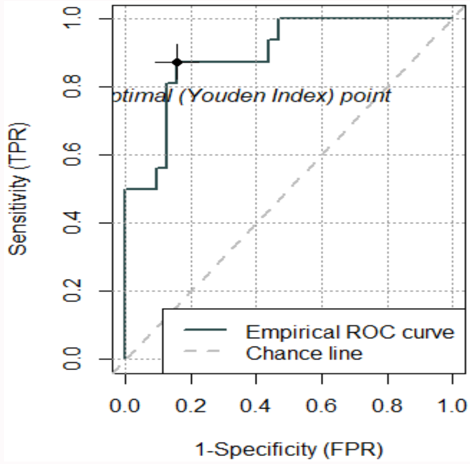

Figure 1: FSH concentration and sensitivity of testicular spermatogenesis.

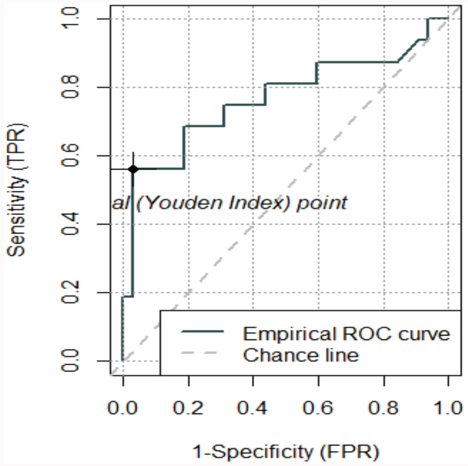

Figure 2: LH concentration and sensitivity of testicular spermatogenesis.

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

	Normal testicle (>TTV 32 cc, n=8) Mean ± SD	Small testicle (<TTV 32 cc, n=40) Mean ± SD	T test p-value
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	3.75 ± 1.61	12.6 ± 10.3	0.0003*
LH	5.8 ± 2.36	7.5 ± 4.24	0.134
Testosterone	5.2 ± 1.51	4.29 ± 1.6	0.172
Prolactin	11.89 ± 3.16	16.27 ± 8.25	0.01

In a multivariate regression analysis, age, infertility period and testosterone level were not associated with factors that may influence successful sperm retrieval. The normal 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.

	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

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

All 32 men with testicular spermatozoa underwent ICSI, and the totals of 197 oocytes were retrieved from 29 women. Numbers 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 9 shows comparison of paternal characteristics between pregnant and non-pregnant women.

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 et al. [16]. As well as, Yiru et al. reported there were 76.0% (n=140) patients aged 30 to 39, while in our study there were 61.1% (n=22) people aged 30 to 39 [17]. 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 [18].

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 [7,19,20]. 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 [21]. Study reported, FSH level, age and testicular volume were included in the prediction model for sperm retrieval failure risk [21].

There are few studies on the relationship between male age and fertility outcome. The evidence regarding its negative impact on fertility has been inconsistent [22-25]. 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 [26]. 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 [27-30]. 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 [30,31]. Nevertheless, a negative correlation was identified between serum testosterone levels and BMI, suggesting a potential impact on fertility [31]. 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 [19,32]. 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 [32,33]. Turunc et al. [34] suggested that FSH could not be used as a factor to predict

Table 9: Comparison of some indicators of men in pregnant and non-pregnant female partners 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
	02-10	7	24.1	13	44.8	
	>10	2	6.9	4	13.8	
FSH	Less than12.4 U/L	6	20.7	17	58.6	0.021
	More than 12.4 U/L	3	10.3	3	10.3	
Testosterone	>2.8 ng/ml	8	28.6	16	57.1	0.102
	<2.8 ng/ml	1	3.8	3	10.7	
Prolactin	<21.4 ng/ml	2	20	5	50	0.25
	>21.4 ng/ml	1	1	2	20	

the success of testicular sperm retrieval in patients with NOA [35]. Kızılay et al. evaluate to the sperm retrieval and factors affecting these rates in men who underwent repeat mTESEs [36]. This study results confirm testicular volume, histology, karyotype, and Y-chromosome microdeletion were predicting factors for successful sperm retrieval. Gnessi also developed a model for predicting outcomes in which younger men and lower FSH levels are important [37]. 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 ± 14.56 mIU/mL, while the successful group had 7.94 ± 4.95 mIU/mL [38]. In our study, it was19.7 ± 12.3 mIU/mL in unsuccessful sperm retrieval cases and 6.77 ± 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 ± 8.43 mIU/mL for obstructive azoospermia and 20.12 ± 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 [39].

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 [40]. 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.

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.

References

1. Ombelet W. WHO fact sheet on infertility gives hope to millions of infertile couples worldwide. Facts Views Vis Obgyn. 2020;12(4):249-51.

2. Stephen EH, Chandra A. Declining estimates of infertility in the United States: 1982-2002. Fertil Steril. 2006;86(3):516-23.

3. Klami R, Mankonen H, Perheentupa A. Successful microdissection testicular sperm extraction for men with non-obstructive azoospermia. Reprod Biol. 2018;18(2):137-42.

4. Cerilli LA, Kuang W, Rogers D. A practical approach to testicular biopsy interpretation for male infertility. Arch Pathol Lab Med. 2010;134(8):1197-204.

5. Wosnitzer M, Goldstein M, Hardy MP. Review of Azoospermia. Spermatogenesis. 2014;4:e28218.

6. Esteves SC, Lee W, Benjamin DJ, Seol B, Verza S Jr., Agarwal A. Reproductive potential of men with obstructive azoospermia undergoing percutaneous sperm retrieval and intracytoplasmic sperm injection according to the cause of obstruction. J Urol. 2013;189(1):232-7.

7. Colpi GM, Caroppo E. Performing microdissection testicular sperm extraction: Surgical pearls from a high-volume infertility center. J Clin Med. 2021;10(19):4296.

8. Dabaja AA, Schlegel PN. Microdissection testicular sperm extraction: An update. Asian J Androl. 2013;15(1):35-9.

9. Saccà A, Pastore AL, Roscigno M, Naspro R, Pellucchi F, Fuschi A, et al. Conventional testicular sperm extraction (TESE) and non-obstructive azoospermia: is there still a chance in the era of microdissection TESE? Results from a single non-academic community hospital. Andrology. 2016;4(3):425-9.

10. Practice Committee of the American Society for Reproductive Medicine in collaboration with the Society for Male Reproduction and Urology. The management of obstructive azoospermia: a committee opinion. Fertil Steril. 2019;111(5):873-80.

11. Caroppo E, Colpi GM. Prediction models for successful sperm retrieval in patients with non-obstructive azoospermia undergoing microdissection testicular sperm extraction: Is there any room for further studies? J Clin Med. 2021;10(23):5538.

12. Majzoub A, Arafa M, Clemens H, Imperial J, Leisegang K, Khalafalla K, et al. A systemic review and meta-analysis exploring the predictors of sperm retrieval in patients with non-obstructive azoospermia and chromosomal abnormalities. Andrologia. 2022;54(3):e14303.

13. Qi L, Liu YP, Zhang NN, Su YC. Predictors of testicular sperm retrieval in patients with non-obstructive azoospermia: A review. J Int Med Res. 2021;49(4):03000605211002703.

14. Jahromi BN, Zeyghami S, Parsanezhad ME, Ghaemmaghami P, Zarei A, Kutenaee MA, et al. Determining an optimal cut-off value for follicle-stimulating hormone to predict microsurgical testicular sperm extraction outcome in patients with non-obstructive azoospermia. Arch Endocrinol Metab. 2020;64(2):165-70.

15. Ming Y, Li M, Dai F, Huang R, Zhang J, Zhang L, et al. Dissecting the current caesarean section rate in Shanghai, China. Sci Rep . 2019;9(1):2080.

16. Chehrazi M, Lanoue J, Ougham K, Moss B, Uthaya S, Modi N. Outcomes in very preterm infants receiving an exclusive human milk diet, or their own mother's milk supplemented with preterm formula. Early Hum Dev. 2023;187:105880.

17. Tsai YR, Lan KC, Kung FT, Lin PY, Chiang HJ, Lin YJ, et al. The effect of advanced paternal age on the outcomes of assisted reproductive techniques among patients with azoospermia using cryopreserved testicular spermatozoa. Taiwan J Obstet Gynecol. 2013;52(3):351-5.

18. Gowri V, Venkiteswaran KP, Al-Zakwani I, Mathew J, Rahman KA, Al-Marhoon M. Comparison of the demographics, semen parameters and hormone profiles in men with primary and secondary infertility. Sultan Qaboos Univ Med J. 2010;10(3):350-3.

19. Tang WH, Zhou SJ, Song SD, He HY, Wu H, Zhang Z, et al. A clinical trial on the consistency of bilateral testicular tissue histopathology and Johnsen score: Single side or bilateral side biopsy? Oncotarget. 2018;9(35):23848-59.

20. Li F, Yang Q, Shi H, Xin H, Luo X, Sun Y. Effects of obesity on sperm retrieval, early embryo quality and clinical outcomes in men with nonobstructive azoospermia undergoing testicular sperm aspiration-intracytoplasmic sperm injection cycles. Andrologia. 2019;51(6):e13265.

21. Ma Y, Li F, Wang L, Zhao W, Li D, Xian Y, et al. A risk prediction model of sperm retrieval failure with fine needle aspiration in males with non-obstructive azoospermia. Hum Reprod. 2019;34(2):200-8.

22. Van Opstal J, Fieuws S, Spiessens C, Soubry A. Male age interferes with embryo growth in IVF treatment. Hum Reprod. 2021;36(1):107-15.

23. Humm KC, Sakkas D. Role of increased male age in IVF and egg donation: is sperm DNA fragmentation responsible? Fertil Steril. 2013;99(1):30-6.

24. Jimbo M, Kunisaki J, Ghaed M, Yu V, Flores HA, Hotaling JM. Fertility in the aging male: A systematic review. Fertil Steril. 2022;118(6):1022-34.

25. Sharma R, Agarwal A, Rohra VK, Assidi M, Abu-Elmagd M, Turki RF.

Effects of increased paternal age on sperm quality, reproductive outcome and associated epigenetic risks to offspring. Reprod Biol Endocrinol. 2015;13:35.

26. Topuz B, Ebiloğu T, Sarıkaya S, Kaya E, Fidan U, Korkmaz C, et al. The timing of micro-TESE: what is the ideal age for male and female partner to bring a child to home? Rev Assoc Med Bras (1992). 2021;67(7):958-65.

27. Bernie AM, Ramasamy R, Schlegel PN. Predictive factors of successful microdissection testicular sperm extraction. Basic Clin Androl. 2013;23:5.

28. Bernie AM, Shah K, Halpern JA, Scovell J, Ramasamy R, Robinson B, et al. Outcomes of microdissection testicular sperm extraction in men with nonobstructive azoospermia due to maturation arrest. Fertil Steril. 2015;104(3):569-73.e1.

29. Ghalayini IF, Al-Ghazo MA, Hani OB, Al-Azab R, Bani-Hani I, Zayed F, et al. Clinical comparison of conventional testicular sperm extraction and microdissection techniques for non-obstructive azoospermia. J Clin Med Res. 2011;3(3):124-31.

30. Ramasamy R, Bryson C, Reifsnyder JE, Neri Q, Palermo GD, Schlegel PN. Overweight men with nonobstructive azoospermia have worse pregnancy outcomes after microdissection testicular sperm extraction. Fertil Steril. 2013;99(2):372-6.

31. Pavan-Jukic D, Starc A, Stubljar D, Jukic T. Obesity with high body mass index does not influence sperm retrieval in males with azoospermia. Med Sci Monit. 2020;26:e923060.

32. Tang WH, Jiang H, Ma LL, Hong K, Zhao LM, Mao JM, et al. [Correlation of testicular volume and reproductive hormone level with the results of testicular sperm aspiration in non-obstructive azoospermia patients]. Zhonghua Nan Ke Xue. 2012;18(1):48-51.

33. Xue H, Wang SY, Cui LG, Hong K. Can contrast-enhanced ultrasound increase or predict the success rate of testicular sperm aspiration in patients with azoospermia? AJR Am J Roentgenol. 2019;212(5):1054-9.

34. Turunc T, Gul U, Haydardedeoglu B, Bal N, Kuzgunbay B, Peskircioglu L, et al. Conventional testicular sperm extraction combined with the microdissection technique in nonobstructive azoospermic patients: A prospective comparative study. Fertil Steril. 2010;94(6):2157-60.

35. Guler I, Erdem M, Erdem A, Demirdağ E, Tunc L, Bozkurt N, et al. Impact of testicular histopathology as a predictor of sperm retrieval and pregnancy outcome in patients with nonobstructive azoospermia: Correlation with clinical and hormonal factors. Andrologia. 2016;48(7):765-73.

36. Kızılay F, Semerci B, Şimşir A, Kalemci S, Altay B. Analysis of factors affecting repeat microdissection testicular sperm extraction outcomes in infertile men. Turk J Urol. 2019;45(Supp. 1):S1-S6.

37. Gnessi L, Scarselli F, Minasi MG, Mariani S, Lubrano C, Basciani S, et al. Testicular histopathology, semen analysis and FSH, predictive value of sperm retrieval: Supportive counseling in case of reoperation after Testicular Sperm Extraction (TESE). BMC Urol. 2018;18(1):63.

38. 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.

39. Ari B, Ponco B, Nur R. Cut-off value of testosterone and FSH level in patient with azoospermia. KnE Medicine. 2016;1(1):145-52.

40. Esteves SC. Microdissection TESE versus conventional TESE for men with nonobstructive azoospermia undergoing sperm retrieval. Int Braz J Urol. 2022;48(3):569-78.