Submitted: 26 March, 2023 Accepted: 23 May, 2023 Published: 30 January, 2024 DOI:10.22514/jomh.2024.008

JOMH
Journal of Men's Health

ORIGINAL RESEARCH

# The predictive value of serum sex hormones for the outcome of testicular sperm aspiration in azoospermia

Yu Yang[1], Mengying Liao[2], Junhua Luo[1], Qunlong Liu[3], Qiao Tian[1], Zhengping Zhao[1], Benlin Wei[1,*]

[1] Department of Urology, Peking University Shenzhen Hospital, 518016 Shenzhen, Guangdong, China
[2] Department of Pathology, Peking University Shenzhen Hospital, 518016 Shenzhen, Guangdong, China
[3] Department of Reproductive Medicine, Peking University Shenzhen Hospital, 518016 Shenzhen, Guangdong, China

***Correspondence**
weibenlin@pkuszh.com
(Benlin Wei)

## Abstract

Azoospermia is a condition that affects the reproductive health of approximately 1% of the male population, typically diagnosed through testicular biopsy. Noninvasive serum markers can play a significant role in the diagnosis of azoospermia diagnosis. This study aims at evaluating the predictive value of serum sex hormone levels to improve the outcome of sperm extraction in azoospermia patients. The data from 203 patients with azoospermia from the Urology and Reproductive Andrology Department of Peking University, Shenzhen Hospital, between January 2017 and March 2022. The cases were divided into the sperm extraction group (95 cases) and the non-sperm extraction group (108 cases), based on pathology results. The levels of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), and inhibin B (INHB) were collected and their predictive effect on the outcome of sperm extraction by testicular sperm aspiration was discussed using statistical analysis. The levels of FSH and LH were lower in the sperm extraction group were lower compared to those in the non-sperm extraction group, while the levels of T and INHB were higher in the sperm extraction group than those in the non-sperm extraction group. These differences were statistically significant ($p < 0.05$). Receiver Operating Characteristic-Area Under the Curve (ROC-AUC) of INHB was 0.950, ROC-AUC of FSH was 0.893, ROC-AUC of LH was 0.78, and that of testosterone was 0.593. The best predictive point of INHB was 94.30 pg/mL, and that of FSH was 10.95 IU/L, calculated by the Youden index. The levels of four serum sex hormones were significantly different between the sperm extraction group and the non-sperm extraction group. The findings suggest that FSH and inhibin B are the ideal serum predictors of the outcome of sperm extraction by testicular sperm aspiration.

## Keywords

Testicular sperm aspiration; Follicle-stimulating hormone; Inhibin B

## 1. Introduction

Azoospermia is a challenging type of male infertility that accounts for approximately 10%–20% of male infertility cases [1, 2]. The etiology of azoospermia includes multiple causes, including pre-testicular factors such as sex hormone abnormalities [3, 4], abnormal testicular function [5, 6], and retrotesticular obstructive factors [7, 8]. Testicular factors include congenital (karyotype abnormality like 47XXY, 45X, anorchia, cryptorchidism, Y-chromosome microdeletion, mosaicism and other genetic abnormalities), germ cell hypoplasia (Sertoli cell-only syndrome), and arrested maturation. Acquired causes of testes include bacterial or viral infection (*i.e.*, mumps orchitis and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viral) [9, 10], trauma and operation, tumors and chemotherapy and radiation therapy, alcohol and drug use, and varicocele [11]. In recent years, assisted reproductive technology (ART) combined with testicular sperm aspiration (TESA) or microscope testicular sperm extraction (Micro-TESE) has significantly improved fertility opportunities for azoospermic patients [12, 13]. The differential diagnosis of obstructive azoospermia (OA) and non-obstructive azoospermia (NOA) is critical for operating sperm retrieval. Micro-TESE exhibits an overall success rate of approximately 50% [14], and remains the most favorite treatment for NOA patients. Testicular biopsy is the classic diagnostic procedure to verify the spermatogenicity and pathological type of testis [13, 15]. However, the procedure can also cause hematoma and inflammation to the remaining spermatogenic ducts and aggravates the stress and psychological burden of patients [16].

Sex hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), and inhibin B (INHB), are reliable indicators of the endocrine function from the hypothalamus-pituitary-testis axis. Therefore, FSH, LH, T and INHB can be applied in the analysis of the functional state of the testicular germ cells. These hormones are considered ideal serum markers for predicting testicular spermatogenic function and the sperm acquisition rate of testicular biopsy [17]. This study aimed at retrospectively analyzing the predictive potency and significance of these sex hormone indicators (FSH, LH, T and INHB) for the assessment of testicular spermatogenic function and the diagnosis of the patients of azoospermia.

## 2. Materials and methods

### 2.1 Patients

Data was collected from patients with azoospermia patients who underwent testicular biopsy in the Department of Urology and Reproductive Center, Peking University Shenzhen Hospital, from January 2017 to March 2022. The inclusion criteria included: (1) Microscopic examination without sperm after 3 consecutive semen centrifugations and (2) Requirement for birth. Meanwhile, the exclusion criteria were (1) Cryptorchidism, (2) Genital and urinary tract infections or severe trauma, (3) Non-ejaculation/retrograde ejaculation, (4) The presence of any karyotype abnormality, such as Y chromosome microdeletions, 47XXY, 45X, and any mosaicism (5) Parotiditis history or Varicocele and (6) The occurrence of any tumor history, drug and chemotherapy history and radiation therapy history.

A total of 203 patients with azoospermia, aged between 19–53 years, with an average age of 30.02 ± 4.97 years, were included in the study. The cases were divided into the sperm acquisition group (95 cases) and the sperm loss group (108 cases) based on testicular biopsy pathology. The average age of the sperm acquisition group was 31.22 ± 5.69 years, while that of the sperm loss group was 30.97 ± 4.94 years. The two groups had no significant difference in age between the two groups ($p > 0.05$) (Table 1). The body mass index (BMI) score of the sperm acquisition group was 23.22 ± 2.25, while that of the sperm loss group was 24.18 ± 3.04. The two groups had no significant difference in BMI score ($p > 0.05$) (Table 1).

### 2.2 Method

The levels of FSH, LH, T, Estradiol (E) and INHB in the morning serum of the patients was measured using Enzyme linked immunosorbent assay. Testicular biopsies were performed on the volumetrically dominant testis using routine disinfection and local anesthesia. A 20 mL syringe was used for the puncture under negative pressure to extract testicular tissue of approximately 5 mm × 5 mm, and a pathological examination was performed after Bouin's fluid fixation.

### 2.3 Data analysis

The data was collected and analyzed using SPSS 28.0 (IBM Corp, Armonk, NY, USA). The normality of the data distribution was analyzed using the Kolmogorov-Smirnov test for normality preceding statistical analysis. The data conforming to the normal distribution were presented as mean ± standard deviation, while non-normally distributed data (not conforming to the normal distribution) was presented as Median (P25, P75). The mean parameters of two groups conforming to the normal distribution were compared by $t$-test and the median parameters of two groups not conforming to the normal distribution were compared by Mann-Whitney U-test, at test level $\alpha = 0.05$. The receiver operating characteristic (ROC) curve was used to evaluate the reliability of candidate indicators as diagnosis biomarkers. The area under the curve (AUC) was used to evaluate the diagnostic and predictive value of relevant indicators. The cut-off value was determined using the Young Index Method.

## 3. Result

### 3.1 Comparison of sex hormone indicators in two groups of patients

The levels of FSH and LH levels in the sperm acquisition group were significantly lower than those in the sperm loss group, while the levels of T and INHB levels were significantly higher than those in the sperm loss group, with statistical significance ($p < 0.05$). There was no significant difference in serum E levels between the two groups ($p = 0.114$) (Table 1).

### 3.2 ROC curve of sex hormone indicators predicting TESA outcome

The ROC curve was plotted using serum FSH, LH, T and INHB levels to predict the outcome of TESA using SPSS28.0 (Figs. 1,2). The ROC-AUC values for INHB, FSH, LH and testosterone were 0.950, 0.893, 0.78 and 0.593, respectively. The Youden index method was used to determine the best predictive values of INHB and FSH, which were 94.30 pg/mL and 10.95 IU/L, respectively (Table 2).

## 4. Discussion

Sex hormone detection has recently become widely adopted for the estimation of male reproductive function due to advances in laboratory technology. The noninvasive nature of the approach

**TABLE 1. Comparison of sex hormone indicators in two groups of patients.**

| Group | Case | Age | FSH (IU/L) | LH (IU/L) | T (ng/mL) | INHB (pg/mL) | E (pg/mL) |
|---|---|---|---|---|---|---|---|
| Sperm Acquisition Group | 95 | 31.22 ± 5.69 | 6.90 (4.50, 10.80) | 4.22 (2.60, 6.21) | 4.02 ± 1.70 | 124.05 (90.57, 165.03) | 30.41 ± 7.10 |
| Sperm Loss Group | 108 | 30.97 ± 4.94 | 21.35 (15.84, 27.75) | 7.17 (5.74, 9.47) | 3.38 ± 1.01 | 23.89 (11.75, 44.52) | 28.24 ± 8.33 |
| $p$-value | | 0.360* | <0.001** | <0.001** | 0.002* | <0.001** | 0.114* |

**: t-test; **: Mann-Whitney U-test; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; INHB: inhibin B; E: estradiol.*

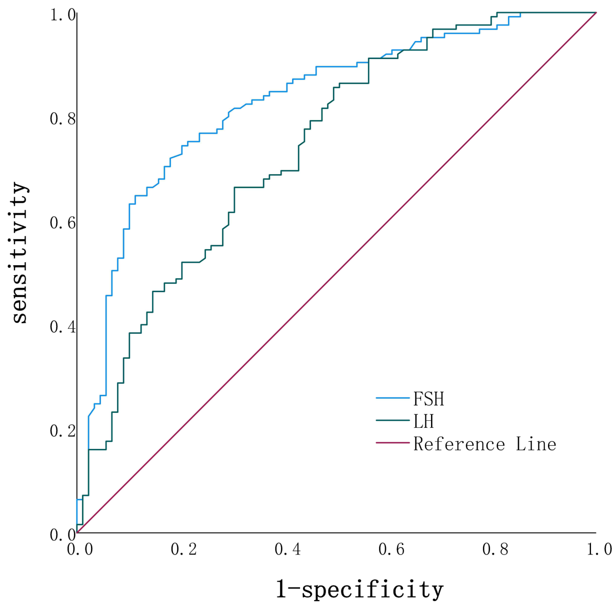


**FIGURE 1. ROC curves of serum FSH and LH illustrated to evaluate the predictive value as indicators of TESA outcome.** FSH: follicle stimulating hormone; LH: luteinizing hormone; T: testosterone; INHB: inhibin B.

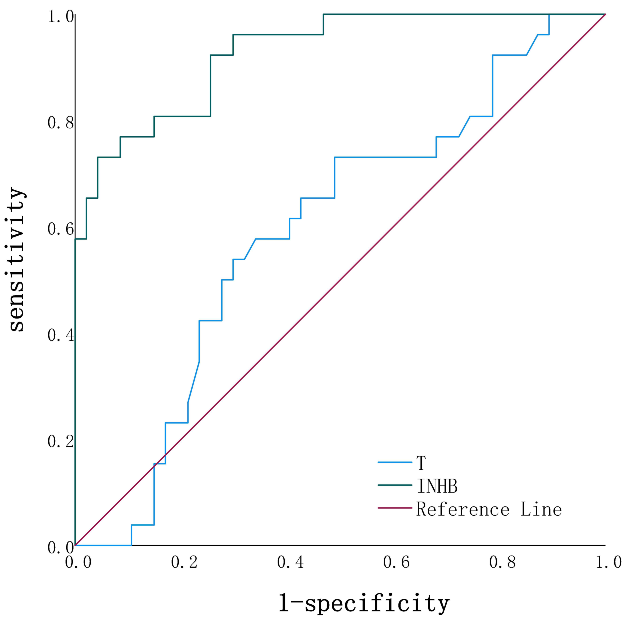


**FIGURE 2. ROC curves of serum T and INHB illustrated to evaluate the predictive value as indicators of TESA outcome.** FSH: follicle stimulating hormone; LH: luteinizing hormone; T: testosterone; INHB: inhibin B.

**TABLE 2. ROC curve of sex hormone indicators predicting TESA outcome.**

| | ROC-AUC | Best cut-off point | Sensitivity | Specificity |
|---|---|---|---|---|
| FSH | 0.893 | 10.95 IU/L | 91.7% | 75.8% |
| LH | 0.782 | 5.68 IU/L | 77.9% | 68.4% |
| T | 0.593 | 3.79 ng/mL | 68.2% | 50.3% |
| INHB | 0.950 | 94.30 pg/mL | 76.9% | 97.3% |

*FSH: follicle stimulating hormone; LH: luteinizing hormone; T: testosterone; INHB: inhibin B; ROC: receiver operating characteristic; AUC: area under the curve.*

is particularly important in evaluating spermatogenic function in azoospermia. Previous studies have shown that FSH was involved in the differentiation and proliferation of all germ cells and directly regulates spermatogenic function. The serum FSH level can potentially affect the spermatogenic function [18, 19] and hence applied for the prediction of the sperm retrieval rate of TESA and Micro-TESE [20, 21]. The best cut-point value of FSH can fluctuate significantly, with values ranging from 5.52–13.31 IU/L across literature [18, 20–22]. The best cut-off point value of FSH in this study is 10.95 IU/L, which is more consistent with most of the literature reports [18, 20–22].

Inhibin B is a negative feedback hormone self-secreted by Sertoli cells, with the potential of specifically reflecting the spermatogenic function without interfering with by the hypothalamus-pituitary-testis axis hormone. Moreover, recent studies have reported Inhibin B as more suitable serum sex hormone indicator to evaluate spermatogenic function [23, 24]. The findings in this study showed that the serum level of INHB had the highest ROC-AUC in predicting the outcome of TESA sperm retrieval, which was consistent with the conclusions reported in the related studies [23, 24]. The best cut-point of INHB for different sperm retrieval procedures varies. Among them, the best cut-off points of INHB for Micro-TESE are reported as 28.55 pg/mL [23] and 40 pg/mL [25], and the best cut-off points for TESA are reported as 67 pg/mL [22]. This latter study also used TESA specimen research, and the best cut-off point value was found around 94.30 pg/mL, with a specificity of 97.3%. The aforementioned findings provide a significant amount of data accumulation and a foundation for further research on the diagnostic value of INHB.

However, the ROC-AUC of the LH and T was less than 0.8, and the specificity of ROC-AUC of testosterone was only 50.3%. The low ROC-AUC of LH and T suggest that these two indicators cannot directly reflect the state of spermatogenic function. The target regulatory cells of LH-T are Leydig cells, that maintain the stability of the testicular spermatogenic microenvironment, but do not directly affect on testicular spermatogenic cells. Therefore, LH-T indicators cannot be used as predictors of the TESA sperm retrieval rate.

## 5. Conclusions

This study aimed at investigating the predictive value of sex hormone levels in 203 azoospermic patients undergoing testicular sperm aspiration (TESA). The results indicated that FSH and INHB had the best predictive value for the outcome of TESA sperm acquisition. Although the findings from this paper suggest relevant application of sex hormone levels in the assessment of sperm extraction viability, some limitations suggest the need for further investigations. These include the small sample size and limited types of sperm retrieval procedures. Therefore, future studies should consider collecting more data, incorporating additional indicators such as testicular volume, and expanding the range of sperm retrieval procedures to further support for developing clinical prediction models.

## AVAILABILITY OF DATA AND MATERIALS

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

## AUTHOR CONTRIBUTIONS

WBL, ZZP and YY—designed the research study. YY and LQL—collected clinical data. LMY—conducted all pathological examination. LJH and TQ—analyzed the data. YY, ZZP and WBL—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethical Committee of Peking University Shenzhen Hospital (no. 202205575). All the patients signed the consent form permitting their data to be used for scientific research under confidentiality of personal information.


## ACKNOWLEDGMENT

Not applicable.

## FUNDING

This work was supported by the Project of Administration of Traditional Chinese Medicine of Guangdong, China (no. 20221014) and Clinical Project of Peking University Shenzhen Hospital (no. LCYJ2020006).


## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES


[1] Esteves SC. Evolution of the World Health Organization semen analysis manual: where are we? Nature Reviews Urology. 2022; 19: 439–446.

[2] Sadeghi MR. A new perspective for the future of male infertility treatment and research. Journal of Reproduction & Infertility. 2020; 21: 1–2.

[3] Lispi M, Drakopoulos P, Spaggiari G, Caprio F, Colacurci N, Simoni M, *et al*. Testosterone serum levels are related to sperm DNA Fragmentation index reduction after FSH administration in males with idiopathic infertility. Biomedicines. 2022; 10: 2599.

[4] Pallotti F, Barbonetti A, Rastrelli G, Santi D, Corona G, Lombardo F. The impact of male factors and their correct and early diagnosis in the infertile couple's pathway: 2021 perspectives. Journal of Endocrinological Investigation. 2022; 45: 1807–1822.

[5] Arumugam M, Shetty DP, Kadandale JS, Kumari SN. Y chromosome microdeletion and cytogenetic findings in male infertility: a cross-sectional descriptive study. International Journal of Reproductive Biomedicine. 2021; 19: 147–156.

[6] Tahmasbpour Marzouni E, Ilkhani H, Beigi Harchegani A, Shafaghatian H, Layali I, Shahriary A. Epigenetic modifications, a new approach to male infertility etiology: a review. International Journal of Fertility & Sterility. 2022; 16: 1–9.

[7] Wang T, Yu Z, Liu Z, Liu X, Li M, Gu L, *et al*. Intra-abdominal robot-assisted vasovasostomy of obstructive azoospermia in an Asian population following multiple bilateral inguinal herniorrhaphy in childhood: a case report and literature review. Translational Andrology and Urology. 2021; 10: 2521–2527.

[8] Cai Z, Li H. Congenital bilateral absence of the vas deferens. Frontiers in Genetics. 2022; 13: 775123.

[9] Teixeira TA, Oliveira YC, Bernardes FS, Kallas EG, Duarte-Neto AN, Esteves SC, *et al*. Viral infections and implications for male reproductive health. Asian Journal of Andrology. 2021; 23: 335–347.

[10] Duarte-Neto AN, Teixeira TA, Caldini EG, Kanamura CT, Gomes-Gouvêa MS, Dos Santos ABG, *et al*. Testicular pathology in fatal COVID-19: a descriptive autopsy study. Andrology. 2022; 10: 13–23.

[11] Pan MM, Hockenberry MS, Kirby EW, Lipshultz LI. Male infertility diagnosis and treatment in the era of *in vitro* fertilization and intracytoplasmic sperm injection. Medical Clinics of North America. 2018; 102: 337–347.

[12] Mazzilli R, Rucci C, Vaiarelli A, Cimadomo D, Ubaldi FM, Foresta C, *et al*. Male factor infertility and assisted reproductive technologies: indications, minimum access criteria and outcomes. Journal of Endocrinological Investigation. 2023; 46: 1079–1085.

[13] Abdel Raheem A, Garaffa G, Rushwan N, De Luca F, Zacharakis E, Abdel Raheem T, *et al*. Testicular histopathology as a predictor of a positive sperm retrieval in men with non-obstructive azoospermia. BJU International. 2013; 111: 492–499.

[14] Punjani N, Kang C, Lee RK, Goldstein M, Li PS. Technological advancements in male infertility microsurgery. Journal of Clinical Medicine. 2021; 10: 4259.

[15] Xia Y, Chen M, Zhu P, Lu C, Fu G, Zhou X, *et al*. Urinary phytoestrogen levels related to idiopathic male infertility in Chinese men. Environment International. 2013; 59: 161–167.

[16] Mantravadi K, Rao D. 46XX testicular disorder of sex development. Journal of Human Reproductive Sciences. 2021; 14: 436–438.

[17] Madian A, Eid MM, Shahin AAB, Mazen I, El-Bassyouni HT, Eid OM. Detection of low-grade mosaicism and its correlation with hormonal profile, testicular volume, and semen quality in a cohort of Egyptian Klinefelter and Klinefelter-like patients. Reproductive Biology. 2020; 20: 259–263.

[18] Chen B, Yang Q, Huang Y, Wang H, Hu K, Wang Y, *et al*. Follicle-stimulating hormone as a predictor for sperm retrieval rate in patients with nonobstructive azoospermia: a systematic review and meta-analysis. Asian Journal of Andrology. 2015; 17: 281–284.

[19] Madani AH, Falahatkar S, Heidarzadeh A, Roshan ZA, Sazgari E, Zareian M. Sensitivity and specificity of serum FSH and testis size in predicting the existence of spermatogenesis in azoospermic infertile men. Andrologia. 2012; 44: 205–209.

[20] 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. Journal of Obstetrics and Gynaecology Research. 2005; 31: 384–388.

[21] Colpi GM, Colpi EM, Piediferro G, Giacchetta D, Gazzano G, Castiglioni FM, *et al*. Microsurgical TESE versus conventional TESE for ICSI in non-obstructive azoospermia: a randomized controlled study. Reproductive BioMedicine Online. 2009; 18: 315–319.

[22] Bonarriba CR, Burgués JP, Vidaña V, Ruiz X, Pizá P. Predictive factors of successful sperm retrieval in azoospermia. Actas UrolóGicas EspañOlas. 2013; 37: 266–272. (In Spanish)

[23] Deng Y, Li D, Hu Y, Chen J, Chu Q. Serum follicle-stimulating hormone in combination with serum inhibin B evaluates spermatogenesis of azoospermic men. Journal of Southern Medical University. 2014; 34: 584–587. (In Chinese)

[24] Chu Q, Hua R, Luo C, Chen Q, Wu B, Quan S, *et al*. Relationship of genetic causes and inhibin B in non obstructive azoospermia spermatogenic failure. BMC Medical Genetics. 2017; 18: 98.

[25] Ballescá JL, Balasch J, Calafell JM, Alvarez R, Fábregues F, de Osaba MJ, *et al*. Serum inhibin B determination is predictive of successful testicular sperm extraction in men with non-obstructive azoospermia. Human Reproduction. 2000; 15: 1734–1738.

**How to cite this article:** Yu Yang, Mengying Liao, Junhua Luo, Qunlong Liu, Qiao Tian, Zhengping Zhao, *et al*. The predictive value of serum sex hormones for the outcome of testicular sperm aspiration in azoospermia. Journal of Men’s Health. 2024; 20(1): 57-61. doi: 10.22514/jomh.2024.008.