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ORIGINAL ARTICLE

Male Infertility

# Predictive significance of serum inhibin B on testicular haploid gamete retrieval outcomes in nonobstructive azoospermic men

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The purpose of this study was to determine the diagnostic accuracy of serum inhibin B (INHB) as a predictor of the retrieval outcome of testicular haploid gametes (spermatids and testicular spermatozoa) in nonobstructive azoospermic men. Serum hormone levels, testicular volume, and histological evaluation were performed in 403 Chinese nonobstructive azoospermic men. Testicular haploid gamete was successfully retrieved in 213 of 403 patients (52.85%). The haploid gamete group always had higher INHB levels than the non-haploid gamete group. According to the receiver operating characteristic (ROC) curve analysis, INHB was a good predictor of testicular haploid gamete retrieval outcome in all patients (sensitivity: 77.93% and specificity: 91.58%) and patients with normal follicle-stimulating hormone (FSH; sensitivity: 88.52% and specificity: 70.83%). The area under the ROC curve (AUC) of INHB was similar to that of FSH in all patients or patients with normal FSH. In patients with elevated FSH, INHB was superior to FSH in predicting the presence of haploid gamete (AUC: 0.73 vs 0.55,  $P < 0.05$ ), with a sensitivity of 60.00% and a specificity of 80.28%. It concluded that serum INHB as an effective marker for spermatogenesis was a significant predictor of testicular haploid gamete retrieval outcomes in nonobstructive azoospermic men. Especially, INHB is superior to FSH in predicting the presence of haploid gamete in the patients with elevated FSH.

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**Keywords:** azoospermia; biomarker; follicle-stimulating hormone; inhibin B; testicular sperm extraction

## INTRODUCTION

The incidence of male infertility is increasing by year, of which azoospermia patients are accounted for about 10%–20%.<sup>1</sup> There are two types of azoospermia: obstructive azoospermia (OA) and nonobstructive azoospermia (NOA). Around 60% of these cases are due to NOA.<sup>2</sup> Since the introduction of intracytoplasmic sperm injection (ICSI) into clinical practice, testicular spermatozoa have made fatherhood a possibility for patients with NOA.<sup>3–5</sup> The sperm retrieval rate of testicular sperm extraction (TESE) or microdissection testicular sperm extraction (micro-TESE) in NOA patients is only about 50%.<sup>6</sup> However, there are no precise clinical biomarkers that can predict the haploid gamete retrieval rate. Finding such an indicator is particularly important, which to some extent can reduce the risk of testicular dysfunction after testicular surgery. In the current clinical practice, serum follicle-stimulating hormone (FSH), testosterone (T), and testicular volume have been commonly used as indicators of sperm retrieval rate in preoperative evaluation, but the clinical value of these parameters is limited.<sup>7–10</sup> FSH levels are easily influenced by gonadotropin-releasing hormone (GnRH), estradiol, and T. Testosterone and testicular volume cannot reflect the presence of focal

spermatogenesis.<sup>7,11</sup> Even if the testicular volume is  $<5$  ml, spermatozoa can also be successfully retrieved from the testis, and the measurement of testicular volume is easily affected by subjective factors.

Serum inhibin B (INHB) is a heterodimeric glycoprotein consisting of an  $\alpha$ -subunit and a  $\beta$ -subunit, and it is possibly a joint product of Sertoli cells and germ cells.<sup>12</sup> The level of INHB is negatively correlated with gonadotropin and FSH in feedback regulation.<sup>12–14</sup> Compared with FSH, INHB should be a more direct and more sensitive predictor of spermatogenesis.<sup>15–17</sup> However, it is still controversial whether serum INHB can predict sperm retrieval outcomes of TESE or micro-TESE in NOA and whether INHB is superior to FSH in predicting the presence of spermatozoa.

In patients who cannot obtain spermatozoa for ICSI, round spermatid injection (ROSI) or elongated spermatid injection (ELSI) after cell cultured is another choice for getting blood descendants.<sup>18–23</sup> However, the testicular haploid gamete (including spermatids and spermatozoa) retrieval rate is rarely reported. In addition, little information is available on predictive factors of successful haploid gamete retrieval in nonobstructive azoospermic men. In this study, we collected the clinical data of 403 patients with NOA in Reproductive

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Hospital Affiliated to Shandong University (Jinan, China), analyzed the predictive value of serum INHB on testicular haploid gamete retrieval outcome, and compared it with other parameters such as serum FSH, luteinizing hormone (LH), T, and testicular volume.

## PATIENTS AND METHODS

### Patients

Data from a total of 403 Chinese nonobstructive azoospermic men who underwent TESE ( $n = 312$ ) or micro-TESE ( $n = 91$ ) were collected from October 2015 to April 2017 in Reproductive Hospital Affiliated to Shandong University. The World Health Organization (WHO) manual for standardized investigation, diagnosis, and management of the infertile male was followed in this study.<sup>24</sup> The azoospermic men were confirmed by at least three semen analyses. All patients underwent detailed medical history acquisition, physical examination, chromosome karyotyping, and azoospermia factor (AZF) microdeletion screening. Testicular volume was measured by ultrasound. Patients with obstructive factors who have sperm retrieved through epididymal puncture were excluded from this study. Patients with chromosomal abnormalities were also not included in this study. This study was approved by the Institutional Review Board (IRB) of Shandong University (Jinan, China). Written informed consent was obtained from the patients at the time of presentation for TESE or micro-TESE.

The patients were categorized into different groups depending on their FSH levels or the histological evaluation of testicular tissue. (1) Patients were categorized into two groups depending on the histological evaluation of testicular tissues (**Table 1**): haploid gamete group ( $n = 213$ ) and non-haploid gamete group ( $n = 190$ ); (2) patients were categorized into four groups depending on their FSH levels and the histological evaluation of testicular tissues (**Table 2**): Group I (normal FSH, haploid gamete), Group II (normal FSH, non-haploid gamete), Group III (elevated FSH, haploid gamete), and Group IV (elevated FSH, non-haploid gamete); (3) patients were categorized into two groups depending on the FSH levels (**Supplementary Table 1**): elevated FSH group ( $n = 172$ ) and normal FSH group ( $n = 231$ ); (4) patients were categorized into two groups depending on testicular histological results

(**Supplementary Table 2**): sperm group ( $n = 196$ ) and azoospermia group ( $n = 207$ ); and (5) patients were categorized into two groups depending on the type of surgical procedure (**Supplementary Table 3**): TESE group ( $n = 312$ ) and micro-TESE group ( $n = 91$ ).

### Physical and laboratory examination

For semen collection and analysis, semen samples were collected by masturbation after 2–7 days of sexual abstinence, and manual semen analysis was performed within 30 min according to the WHO guidelines.<sup>24</sup> Each patient had his semen analyzed at least three times.

To determine the reproductive hormones, venous blood samples were obtained from the antecubital veins between 8:00 a.m. and 10:00 a.m. Serum samples were separated and assayed for INHB, FSH, LH, and T. The serum INHB concentration was measured by the DS2 automatic immunoassay analyzer (DYNEX Technologies Inc., Chantilly, VA, USA), using the INHB enzyme-linked immunosorbent assay (ELISA) kit (Guangzhou Kang Run Biotech Co., Ltd., Guangdong, China). The detection range of the assay was 10–1500 pg ml<sup>-1</sup>, and the intra- and inter-assay coefficient of variation was ≤10% and ≤15%, respectively. The minimum detectable concentration for INHB was 10 pg ml<sup>-1</sup>. The serum FSH, LH, and T concentrations were measured by the Cobas e601 automatic electrochemical luminescence immunoassay system (F. Hoffmann-La Roche Ltd., Basel, Switzerland) according to the manufacturer's instructions. The normal ranges established in our laboratory were as follows: INHB: 54–235 pg ml<sup>-1</sup>, FSH: 1.5–12.4 IU l<sup>-1</sup>, LH: 1.7–8.6 IU l<sup>-1</sup>, and T: 249–836 ng dl<sup>-1</sup>.

During testicular assessment, ultrasound measurement was repeated three times for each testis and the average volume was taken. Testicular tissue was sent to the pathology laboratory for the histological evaluation after TESE or micro-TESE.

### Statistical analyses

Data were analyzed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) and MedCalc version 15.2 (MEDCALC Inc., Ostend, Belgium), and expressed as median and quartile range. Differences of parameters between groups were compared using the Mann–Whitney U test. The

**Table 1: Age, reproductive hormones, and testicular volume differences between groups, which were grouped according to testicular histological results, with Mann–Whitney U test**

Variable	Haploid gamete group ( $n=213$ )	Non-haploid gamete group ( $n=190$ )	<i>P</i>
Age (year)	29.91 (26.00, 32.00)	28.66 (26.00, 31.00)	0.052
INHB (pg ml <sup>-1</sup> )	112.53 (79.01, 146.07)	28.96 (11.00, 30.62)	<0.001
FSH (IU l <sup>-1</sup> )	7.06 (3.56, 7.49)	18.02 (12.40, 21.67)	<0.001
LH (IU l <sup>-1</sup> )	7.43 (3.29, 6.79)	13.56 (6.03, 11.30)	<0.001
T (ng dl <sup>-1</sup> )	500.67 (353.70, 638.00)	364.88 (260.00, 446.25)	<0.001
Testicular volume (ml)	13.70 (12.00, 15.00)	10.83 (8.49, 12.30)	<0.001

Data were shown as median (quartile range). INHB: inhibin B; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone

**Table 2: Age, reproductive hormones, and testicular volume differences between groups, which were grouped according to follicle-stimulating hormone level and testicular histopathological results**

Variable	Group I ( $n=183$ )	Group II ( $n=48$ )	<sup>a</sup> <i>P</i>	Group III ( $n=30$ )	Group IV ( $n=142$ )	<sup>b</sup> <i>P</i>
Age (year)	30.02 (26.00, 32.00)	27.85 (26.00, 29.00)	0.031	29.20 (27.00, 31.00)	28.93 (26.00, 31.00)	0.610
INHB (pg ml <sup>-1</sup> )	125.90 (95.79, 154.33)	61.05 (13.30, 97.64)	<0.001	30.97 (14.13, 44.88)	18.11 (10.50, 17.67)	<0.001
FSH (IU l <sup>-1</sup> )	5.00 (3.38, 6.25)	8.34 (5.16, 11.35)	<0.001	19.60 (14.87, 22.68)	21.29 (15.92, 23.63)	0.365
LH (IU l <sup>-1</sup> )	7.19 (3.25, 6.24)	5.73 (3.19, 7.16)	0.001	8.89 (6.97, 9.95)	16.20 (7.62, 11.90)	0.058
T (ng dl <sup>-1</sup> )	514.68 (380.30, 646.00)	402.26 (228.80, 491.35)	<0.001	415.17 (275.00, 518.93)	352.25 (251.45, 437.50)	0.157
Testicular volume (ml)	14.17 (12.00, 15.10)	12.51 (9.10, 13.87)	<0.001	10.78 (8.50, 12.38)	10.27 (8.10, 12.00)	0.255

Data were shown as median (quartile range). Group I: normal FSH, haploid gamete; Group II: normal FSH, non-haploid gamete; Group III: elevated FSH, haploid gamete; and Group IV: elevated FSH, non-haploid gamete. <sup>a</sup>Group I versus Group II, <sup>b</sup>Group III versus Group IV, with Mann–Whitney U test. INHB: inhibin B; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone

receiver operating characteristic (ROC) curve was used to analyze the predictive value of each parameter for the testicular haploid gamete retrieval outcomes. The area under the ROC curve (AUC) indicated predictive power (low predictive power:  $0.50 < \text{AUC} \leq 0.70$ , medium predictive power:  $0.70 < \text{AUC} \leq 0.90$ , and high predictive power:  $0.9 < \text{AUC} \leq 1.0$ ).  $P < 0.05$  indicated statistical significance.

## RESULTS

The haploid gamete retrieval rates were 52.85%, 79.22%, and 17.44% in all patients, the normal FSH group, and the elevated FSH group, respectively. All parameters except age had significantly different values in the haploid gamete group and the non-haploid gamete group in all patients (Table 1) or patients with normal FSH (Table 2) (all  $P \leq 0.001$ ). In the patients with elevated FSH, only serum INHB was significantly higher in the haploid gamete group than that in the non-haploid gamete group (Table 2;  $P < 0.001$ ).

To evaluate the predictive values of serum INHB, FSH, LH, T, and testicular volume for the retrieval outcome of testicular haploid gamete, the ROC curve was established on the basis of the histological evaluation of testicular tissue taken to be “gold standard.” The cutoff value corresponding to the maximum Youden index was selected as the best diagnostic threshold. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. In all patients or patients with normal FSH, the AUC of serum INHB was similar to that of FSH ( $P > 0.05$ ). However, INHB and FSH performed better than T, LH, and testicular volume in predicting the presence of haploid gamete (all  $P < 0.05$ ). In all patients, the best discriminating cutoff value of INHB for the retrieval outcomes of testicular haploid

gamete was  $68.53 \text{ pg ml}^{-1}$  (AUC: 0.90, sensitivity: 77.93%, specificity: 91.58%, PPV: 91.21%, and NPV: 78.73%) and that of FSH was  $9.00 \text{ IU l}^{-1}$  (AUC: 0.87, PPV: 90.05%, and NPV: 80.66%) (Table 3 and Figure 1). In patients with elevated FSH, the predictive value of INHB for the retrieval outcome of testicular haploid gamete was better than that of FSH and other parameters (LH, T, and testicular volume) ( $P < 0.05$ ), and the best discriminating cutoff value of INHB was  $20.38 \text{ pg ml}^{-1}$  (AUC: 0.73, sensitivity: 60.00%, and specificity: 80.28%; Table 4). Owing to the low haploid gamete retrieval rate (17.44%) in these patients, PPV was only 39.13% and NPV was 90.48%. In patients with normal FSH, the best discriminating cutoff value of INHB was  $75.64 \text{ pg ml}^{-1}$  (AUC: 0.82, sensitivity: 88.52%, specificity: 70.83%, PPV: 92.04%, and NPV: 61.81%) and that of FSH was  $7.51 \text{ IU l}^{-1}$  (AUC: 0.79, PPV: 91.01%, and NPV: 60.37%) (Table 5).

In addition, we observed the predictive value of parameters in different types of surgical procedure (TESE and micro-TESE; Supplementary Table 4 and 5). We also performed the predictive significance of INHB and other parameters on testicular sperm retrieval outcomes (Supplementary Table 6). The predictive value of INHB was predominately stable in different situations.

## DISCUSSION

In this study, our data showed significant differences in the mean levels of INHB and FSH between the haploid gamete group and the non-haploid gamete group in all patients, which was similar to those found in most previous studies.<sup>25–29</sup> However, the majority of these studies were to investigate diagnostic accuracy on TESE outcomes. In our study, the successful surgical outcomes included obtaining

**Table 3: Diagnostic accuracy of reproductive hormones and testicular volume for the retrieval outcomes of testicular haploid gamete in all patients**

Variable	AUC (95% CI)	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
INHB	0.90 (0.86–0.92) <sup>a</sup>	>68.53	77.93	91.58	91.21	78.73
FSH	0.87 (0.83–0.90) <sup>b</sup>	≤9.00	80.75	90.00	90.05	80.66
LH	0.79 (0.75–0.83) <sup>c</sup>	≤7.84	86.85	60.00	70.88	80.28
T	0.71 (0.66–0.75)	>391.30	68.08	64.74	68.40	64.41
Testicular volume	0.76 (0.72–0.80)	>11.9	83.10	65.79	73.14	77.64

<sup>a</sup> $P < 0.05$ : INHB versus LH, INHB versus T, INHB versus testicular volume; <sup>b</sup> $P < 0.05$ : FSH versus LH, FSH versus T, FSH versus testicular volume; <sup>c</sup> $P < 0.05$ : LH versus T. AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; INHB: inhibin B; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone

**Table 4: Diagnostic accuracy of reproductive hormones and testicular volume for the retrieval outcomes of testicular haploid gamete in patients with elevated follicle-stimulating hormone**

Variable	AUC (95% CI)	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
INHB	0.73 (0.66–0.80) <sup>a</sup>	>20.38	60.00	80.28	39.13	90.48
FSH	0.55 (0.48–0.63)	≤15.45	36.67	78.17	26.19	85.39
LH	0.61 (0.53–0.68)	≤10.02	80.00	44.37	23.30	91.30
T	0.58 (0.50–0.66)	>563.00	23.33	94.37	46.68	85.35
Testicular volume	0.57 (0.49–0.64)	>10.97	53.33	66.90	25.39	87.16

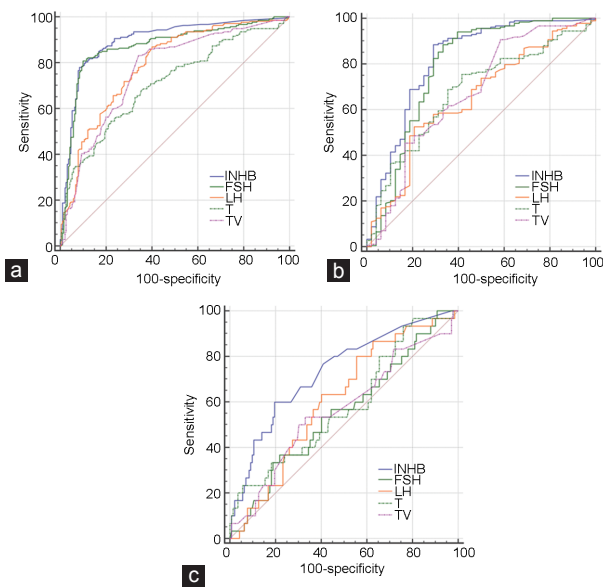
<sup>a</sup> $P < 0.05$ : INHB versus FSH, INHB versus T, INHB versus testicular volume. AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; INHB: inhibin B; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone

**Table 5: Diagnostic accuracy of reproductive hormones and testicular volume for the retrieval outcomes of testicular haploid gamete in patients with normal follicle-stimulating hormone**

Variable	AUC (95% CI)	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
INHB	0.82 (0.76–0.87) <sup>a</sup>	>75.64	88.52	70.83	92.04	61.81
FSH	0.79 (0.73–0.84) <sup>b</sup>	≤7.51	88.52	66.67	91.01	60.37
LH	0.65 (0.59–0.71)	≤4.39	52.46	79.17	90.57	30.40
T	0.69 (0.61–0.74)	>379.00	75.41	58.33	87.34	38.36
Testicular volume	0.67 (0.61–0.73)	>11.35	90.71	41.67	85.57	54.06

<sup>a</sup> $P < 0.05$ : INHB versus LH, INHB versus T, INHB versus testicular volume; <sup>b</sup> $P < 0.05$ : FSH versus LH, FSH versus testicular volume. AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; INHB: inhibin B; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone





**Figure 1:** ROC curves of reproductive hormones and testicular volume for the retrieval outcomes of testicular haploid gamete. The ROC curves were used to describe the diagnostic accuracy of reproductive hormones and testicular volume for the retrieval outcomes of testicular haploid gamete. In (a) all patients or (b) patients with normal FSH, the area under the ROC curve of INHB was similar to that of FSH ( $P > 0.05$ ). However, both of them performed better than T, LH, and TV (all  $P < 0.05$ ). (c) In patients with elevated FSH, the predictive value of INHB for the retrieval outcome of testicular haploid gamete was better than that of FSH and other parameters (LH, T, and TV) ( $P < 0.05$ ). ROC: receiver operating characteristic; INHB: inhibin B; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; TV: testicular volume.

not only testicular spermatozoa but also testicular spermatids. This is one of the largest differences with other studies.<sup>1,2,11,13,15,16,25–29</sup> For this reason, the haploid gamete (including spermatid and testicular spermatozoa) retrieval rate (52.85%) was slightly higher than those in these studies (about 50%). In addition, LH, T, and testicular volume also had statistical difference between groups. It is noteworthy that only serum INHB was significantly higher in the haploid gamete group than that in the non-haploid gamete group ( $P < 0.001$ ) in patients with elevated FSH. In these patients, the FSH level and other parameters had no statistical difference between groups. This suggests that these parameters (FSH, LH, T, and testicular volume) may not predict the presence of haploid gametes in this special group of patients. The proportion of patients with elevated FSH was 42.68% (172/403) of the total patients, but the haploid gamete retrieval rate was only 17.44%. Therefore, for them, a noninvasive marker, predicting successful haploid gamete retrieval before surgery, would be more urgent.

To further analyze the diagnostic accuracy of each parameter as a predictor of the testicular haploid gamete retrieval outcomes, we regarded the histological results of testicular tissue as the “gold standard” to establish the ROC curve. In total patients or patients with normal FSH, serum INHB all had a good sensitivity and specificity for predicting the testicular haploid gamete retrieval outcome in nonobstructive azoospermic men. Different from Huang’s study<sup>29</sup> (sensitivity: 83.5%, specificity: 79.1%, and AUC: 0.862), the present study showed a lower sensitivity (77.93%), with a higher specificity (91.58%) and a higher AUC (0.90) in total patients. Ballesca *et al.*<sup>25</sup> demonstrated that the AUC of INHB was 0.98, with the sensitivity of 90% and the specificity of 100%. However, this study included only

17 NOA patients who underwent TESE, which limits the significance of these data. The AUC of serum INHB was similar to that of FSH in total patients or patients with normal FSH, which is in accordance with some previous reports.<sup>29–31</sup> Serum INHB was not a more sensitive predictor of successful haploid gamete retrieval outcome than FSH in total patients or patients with normal FSH. Although LH, T, and testicular volume had acceptable AUC (0.65–0.79), INHB and FSH were obviously superior to them in predicting the presence of haploid gamete ( $P < 0.05$ ).

However, in patients with elevated FSH, the AUC of INHB was larger than that of FSH ( $P < 0.05$ ), and the best discriminating cutoff value of INHB was 20.38 pg ml<sup>-1</sup> (AUC: 0.73, sensitivity: 60.00%, and specificity: 80.28%), with a NPV of 90.48% and a PPV of 39.13%. The reason of the low PPV may be that the haploid gamete retrieval rate in patients with elevated FSH was only 17.44%, and most of these patients have a strong intention of having surgery. In these patients, the AUC of FSH, LH, T, and testicular volume was  $< 0.70$ , which indicated low predictive power. The AUC of INHB was 0.73, which indicated medium predictive power (AUC: 0.70–0.90). It is undeniable that the diagnostic accuracy of serum INHB was also limited in patients with elevated FSH, but INHB, which was better than FSH, was the best biomarker which we can choose. Under this diagnostic threshold (20.38 pg ml<sup>-1</sup>) of serum INHB, with a high PPV (90.48%), we can effectively exclude patients who are not recommended to undergo surgery and reduce the potential surgical risks.

When we observed the predictive values of parameters in different types of surgical procedure and performed the predictive significance of these parameters on testicular sperm retrieval outcomes, the predictive value of INHB was predominately stable in different situations. In patients who underwent micro-TESE, the diagnostic accuracy of INHB for the retrieval outcome of haploid gamete declined slightly. However, the technique of micro-TESE was only recently employed. When the doctor selected patients for this surgery, it may result in selection bias. Moreover, the proportion of patients who underwent micro-TESE was small.

In 1995, ROSI and ELSI after germ cell culture were reported by Fishel *et al.*<sup>18</sup> and Tesarik *et al.*,<sup>19</sup> respectively. This was another choice for acquiring blood descendants, but owing to the low success rate of ELSI and ROSI in particular, most assisted reproductive medical centers have discontinued the clinical application of these techniques in the mid-1990s. With advances in assisted reproductive and culture *in vitro* techniques, ROSI and ELSI still have huge potential in clinical applications. In 2002, Sousa *et al.*<sup>22</sup> showed that the fertilization rate by ELSI was 48.4%, and the pregnancy rate of women reached 28.9%. In 2015, Tanaka *et al.*<sup>23</sup> reported the health status of 14 infants born at their center through ROSI between September 2011 and March 2014, and none of these infants showed any physical or psychological problems.

Now, little information is available on predictive factors of successful haploid gamete retrieval in nonobstructive azoospermic men, so we conducted this research. The downside was that the proportion of patients who underwent micro-TESE was small. This is because this technique has been applied at our center just for a short time. Micro-TESE is now one of the more popular sperm retrieval techniques for men with NOA. As the number of patients who undergo micro-TESE increases, we may have new findings in the future.

From the previous studies, researchers need to pay attention to the following points when comparing different studies or conducting new research. First, age affects hormonal levels. INHB shows an inverse U-shaped dependence on age, whereas FSH shows a U-shaped dependence on age (optimum 20–40 years).<sup>32</sup> In addition, INHB

showed a highly significantly negative correlation with age in patients older than 30 years.<sup>32</sup> Second, the time of blood sampling may influence hormonal concentrations. INHB concentrations were higher in samples collected in the morning than those collected in the afternoon.<sup>33,34</sup> Jorgensen *et al.*<sup>34</sup> reported that, from 8:00 a.m. to 12:00 a.m., INHB fell to 92%, and in the following 4 h, the hormone level decreased further to 79%. Jorgensen *et al.*<sup>34</sup> also demonstrated that FSH level was not affected by the time of blood sampling, and season was not significantly associated with INHB or FSH level. However, Meeker *et al.*<sup>33</sup> considered that INHB levels were lower in men who had blood samples collected in winter than in men with samples collected in the spring, summer, or fall. Last, researchers should pay attention to whether or not patients with karyotype abnormalities or AZF microdeletions, and patients who received hormone therapy within 3 months of the prestudy assessments were excluded. Y chromosome microdeletions are the second most common genetic reason for male infertility, and this microdeletion often occurs in the AZF region.<sup>35,36</sup> AZF a/b microdeletion often portends the failure of sperm retrieval.<sup>37–39</sup> Patients with AZF a/b microdeletion are not recommended to undergo surgery. Hormone therapy will affect the basic levels of hormones, influencing the credibility of the study. The above factors will affect the comparability of different studies.

## CONCLUSIONS

Serum INHB as an effective marker for spermatogenesis is a significant predictor of testicular haploid gamete retrieval outcome in nonobstructive azoospermic men. Especially, INHB was superior to FSH in predicting the presence of haploid gamete in the patients with elevated FSH.

## AUTHOR CONTRIBUTIONS

ZG Zhu and XJX participated in the design of the study, analyzed data, and wrote the manuscript. ZG Zhao participated in its design and coordination and helped to draft the manuscript. XJX modified the paper. QYP and TC participated in data collection and analyzed data. QYP, TJZ, CX, and XJX performed testicular assessment and surgery. JMZ performed the statistical analysis and helped to draft the manuscript. HBZ participated in data collection. WL performed pathological analysis. All authors read and approved the final manuscript.

## COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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**Supplementary Table 1: Age, reproductive hormones, and testicular volume differences between groups, which were grouped according to follicle-stimulating hormone level, with Mann–Whitney U-test**

	Normal FSH group (n=231)	Elevated FSH group (n=172)	P
Age (year)	28.00 (26.00, 31.00)	28.00 (26.00, 31.00)	0.552
INHB (pg ml <sup>-1</sup> )	111.12 (79.84, 145.03)	13.60 (11.00, 21.85)	<0.001
LH (IU l <sup>-1</sup> )	4.82 (3.26, 6.32)	9.25 (7.25, 11.50)	<0.001
T (ng dl <sup>-1</sup> )	466.00 (351.00, 634.70)	331.85 (255.75, 444.80)	<0.001
Testicular volume (ml)	13.20 (12.00, 15.00)	10.00 (8.23, 12.00)	<0.001

Data were shown as median (upper quartile, lower quartile). INHB: inhibin B; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone

**Supplementary Table 2: Age, reproductive hormones, and testicular volume differences between groups, which were grouped according to testicular histological results, with Mann–Whitney U-test**

	Sperm group (n=196)	Azoospermia group (n=207)	P
Age (year)	28.00 (26.00, 32.00)	28.00 (26.00, 31.00)	0.199
INHB (pg ml <sup>-1</sup> )	111.86 (84.83, 152.66)	14.30 (11.00, 37.34)	<0.001
FSH (IU l <sup>-1</sup> )	5.06 (3.53, 6.85)	16.34 (11.91, 21.57)	<0.001
LH (IU l <sup>-1</sup> )	4.83 (3.29, 6.59)	8.52 (6.00, 11.30)	<0.001
T (ng dl <sup>-1</sup> )	483.20 (354.50, 639.00)	349.00 (262.00, 453.60)	<0.001
Testicular volume (ml)	13.20 (12.00, 15.00)	10.30 (8.60, 12.60)	<0.001

Data were shown as median (upper quartile, lower quartile). INHB: inhibin B; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone

**Supplementary Table 3: Age, reproductive hormones, and testicular volume differences between groups, which were grouped according to the type of surgical procedure, with Mann–Whitney U-test**

	TESE (n=312)	Micro-TESE (n=91)	P
Age (year)	28.00 (26.00, 31.00)	29.00 (27.00, 33.00)	0.043
INHB (pg ml <sup>-1</sup> )	55.54 (12.32, 120.53)	38.84 (14.68, 109.89)	0.938
FSH (IU l <sup>-1</sup> )	9.42 (4.76, 17.77)	9.41 (4.74, 17.06)	0.998
LH (IU l <sup>-1</sup> )	6.31 (4.16, 8.91)	6.57 (4.35, 9.37)	0.782
T (ng dl <sup>-1</sup> )	426.00 (306.00, 563.00)	362.00 (275.00, 509.28)	0.011
Testicular volume (ml)	12.00 (10.00, 13.96)	13.70 (10.99, 15.90)	<0.001

Data were shown as median (upper quartile, lower quartile). INHB: inhibin B; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; TESE: testicular sperm extraction

**Supplementary Table 4: Diagnostic accuracy of reproductive hormones and testicular volume for the retrieval outcomes of haploid gamete in patients who underwent testicular sperm extraction**

	AUC (95% CI)	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
INHB (pg ml <sup>-1</sup> )	0.91 (0.87–0.94) <sup>a</sup>	>75.67	80.86	93.29	92.34	82.98
FSH (IU l <sup>-1</sup> )	0.89 (0.85–0.93) <sup>b</sup>	≤9.19	84.57	91.28	90.65	85.54
LH (IU l <sup>-1</sup> )	0.80 (0.75–0.84)	≤7.84	90.12	61.74	70.20	86.20
T (ng dl <sup>-1</sup> )	0.75 (0.70–0.80)	>458.00	61.11	79.19	74.60	67.06
Testicular volume (ml)	0.77 (0.72–0.82)	>11.90	82.10	69.80	73.11	79.59

<sup>a</sup>P<0.05: INHB versus LH, INHB versus T, INHB versus testicular volume; <sup>b</sup>P<0.05: FSH versus LH, FSH versus T, FSH versus testicular volume. TESE: testicular sperm extraction; AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; INHB: inhibin B; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone

**Supplementary Table 5: Diagnostic accuracy of reproductive hormones and testicular volume for the retrieval outcomes of haploid gamete in patients who underwent microdissection testicular sperm extraction**

	AUC (95% CI)	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
INHB (pg ml <sup>-1</sup> )	0.84 (0.75–0.91) <sup>a</sup>	>43.40	76.47	87.80	86.24	78.86
FSH (IU l <sup>-1</sup> )	0.80 (0.70–0.88) <sup>b</sup>	≤6.75	72.55	92.68	90.84	77.15
LH (IU l <sup>-1</sup> )	0.76 (0.66–0.85) <sup>c</sup>	≤4.97	54.90	90.24	84.91	66.68
T (ng dl <sup>-1</sup> )	0.58 (0.47–0.68) <sup>d</sup>	>399.50	49.02	70.73	62.61	58.11
Testicular volume (ml)	0.74 (0.64–0.83)	>11.00	92.16	48.78	64.28	86.15

<sup>a</sup>P<0.05: INHB versus T, INHB versus testicular volume; <sup>b</sup>P<0.05: FSH versus T; <sup>c</sup>P<0.05: LH versus T; <sup>d</sup>P<0.05: T versus testicular volume. micro-TESE: microdissection testicular sperm extraction; AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; INHB: inhibin B; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; CI: confidence interval

**Supplementary Table 6: Diagnostic accuracy of reproductive hormones and testicular volume for the retrieval outcomes of testicular sperm in all patients**

	<i>AUC (95% CI)</i>	<i>Cutoff value</i>	<i>Sensitivity (%)</i>	<i>Specificity (%)</i>	<i>PPV (%)</i>	<i>NPV (%)</i>
INHB (pg ml <sup>-1</sup> )	0.89 (0.86–0.92) <sup>a</sup>	>54.20	85.20	85.02	85.05	85.17
FSH (IU l <sup>-1</sup> )	0.88 (0.84–0.91) <sup>b</sup>	≤8.80	83.67	87.44	86.95	84.26
LH (IU l <sup>-1</sup> )	0.80 (0.76–0.84) <sup>c</sup>	≤7.84	89.29	58.45	68.24	84.51
T (ng dl <sup>-1</sup> )	0.71 (0.66–0.75)	>391.30	69.39	63.29	65.40	67.40
Testicular volume (ml)	0.75 (0.70–0.79)	>11.90	83.67	62.32	68.95	79.24

<sup>a</sup>*P*<0.05: INHB versus LH, INHB versus T, INHB versus testicular volume; <sup>b</sup>*P*<0.05: FSH versus LH, FSH versus T, FSH versus testicular volume; <sup>c</sup>*P*<0.05: LH versus T. AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; INHB: inhibin B; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; CI: confidence interval