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This article has been updated with language corrections.

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# Value of Serum Inhibin B as a Prognostic Factor in Patients with Non-obstructive Azoospermia

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## Abstract

**Background:** Non-obstructive Azoospermia (NOA) means the absence of sperm in the ejaculate due to testicular failure. Currently, the sole treatment for these patients is testicular sperm retrieval combined with intracytoplasmic sperm injection (ICSI). There is no single test that can precisely predict the presence of sperm in the testis. Several studies have hypothesized that testicular hormones, particularly inhibin B, can aid in the decision-making process for sperm retrieval. The objective of this study was to evaluate the efficacy of serum inhibin B levels in patients with non-obstructive azoospermia as a predictor for successful sperm retrieval using testicular sperm extraction (TESE).

**Methods:** This cross-sectional study carried out on 188 men with non-obstructive azoospermia. A detailed medical history, physical examination, age, duration of infertility and BMI was recorded. Men with obstructive azoospermia were excluded from the study. About 5 ml of venous blood taken from them to measure serum inhibin B, FSH, LH, Prolactin and Testosterone before doing testicular sperm retrieval.

**Results:** From the studied sample, (45.7%) were positive testicular sperm retrieval, while (54.3%) were negative sperm retrieval. The median inhibin B (INHB) level for the TESE-positive group was 54.7 pg/ml, which is significantly higher than that for the TESE-negative group (24 pg/ml). A Receiver Operating Characteristic (ROC) curve analysis of INHB values identified an optimal cutoff of 24 pg/ml, yielding a sensitivity of 70.9% and a specificity of 50%.

**Conclusion:** Serum inhibin B is significantly higher in TESE-positive patients, but its sensitivity and specificity are not sufficient to reliably predict the presence of sperm.

## Introduction

It is estimated that 1% of men and 15% of the infertile men have azoospermia, the cause of azoospermia is either obstructive or non-obstructive [1]. Many factors are responsible for male infertility, including testicular failure, genetic and chromosomal abnormalities, hormonal disturbances, varicocele, genital tract infection, retrograde ejaculation, smoking, alcohol, heavy metal, chemical and radiological exposure, nutritional and different environmental factors that directly or indirectly affect male fertility [2]. Non-obstructive azoospermia (NOA) is defined as the absence of sperm in the ejaculate due to spermatogenic failure and is regarded as the most severe form of male infertility [3]. Testicular sperm retrieval remains the final hope for infertile men with NOA to become biological fathers to their own offspring, serving as an alternative to sperm donation. First pregnancy using surgically retrieved testicular sperm (TESE) from men with non-obstructive azoospermia (NOA) were reported in 1994 [4]. The sperm retrieval rate does not exceed 60% even in the best circumstances [3, 5]. In recent years, non-invasive methods can predict spermatogenesis in patients with non-obstructive azoospermia (NOA) such as measurement of Inhibin B alone or in combination with other hormones [6].Inhibin B is a dimeric glycoprotein hormone secreted from Sertoli cells in response to FSH and act by negative feedback effect on FSH, the secretion of this hormone correlates with the degree of spermatogenesis [7-10].

## Methods

This was observational cross-sectional study carried out on men with non-obstructive azoospermia due to primary testicular failure who seek for fertility treatment in the fertility center of "Alsader medical city", from July, 2022 to April, 2023. The study sample included 188 men, all of whom had non-obstructive azoospermia. Patients were diagnosed by a urologist based on medical history, clinical examination, and investigations such as seminal fluid analysis, hormonal profile (FSH, LH, Prolactin, Testosterone), and testicular size by ultrasound. The ages range was between (20-54) years with mean ± SD (33.4 ± 7.5) years, duration of infertility ranges from (1-30) years with mean ± SD (7.6 years ± 5.5) and BMI range (18.02-40.9) kg/m$^2$ with mean ± SD (26.6 kg/m$^2$ ± 4.7). These patients were recruited for a diagnostic and therapeutic testicular biopsy (TESE, microTESE, or TESA) under spinal or general anesthesia for future fertility treatment via ICSI. In the morning of the surgery a blood sample was taken from all patients at about (9 am-11 am) followed by testicular sperm retrieval surgery.Men who diagnosed with obstructive azoospermia were excluded from the study.

### Blood Samples Collection

For each participant, 5 ml of venous blood was collected from the cubital vein into a gel tube and left at room temperature for at least 30 minutes, then centrifuged at 1000×g force for 10 minutes to separate serum from the blood. The serum is transferred to Eppendorf tube by sterile pipette to be stored at a deep freezer (-80 °C) for future analysis of serum Inhibin B by ELISA system for all the participants in one time.

### Testicular sperm retrieval by testicular Sperm Extraction (TESE)

The technique was performed under general or spinal anesthesia by urologist, all collected samples placed in petri dish containing sterile HEPES buffered media from Vitrolife® in the operating room then transferred to andrology lab. and mechanically minced by two curved needles in aseptic conditions in order to squeeze the spermatozoa out from the seminiferous tubules, no enzymes used in this technique. A wet preparation was then made by placing 10 µl of the sample on a glass slide, covering it with a coverslip, and examining it under a light microscope (40x magnification) to determine the presence of spermatozoa.. If there is no sperm found in the 12 field the specimen is centrifuged (2000 rpm for 10 minutes) and check the pellet if no spermatozoa found in the pellet the specimen considered as negative for sperm.

### INHB test principle

The ELISA kit from Elabscience® utilizes the Sandwich-ELISA principle. This kit does not require sample pretreatment. total incubation time of less than 2 hr. The micro-ELISA plate in this kit is pre-coated with an antibody specific to Human INHB. Samples are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated finding antibody specific for Human INHB and Avidin-Horseradish Peroxidase (HRP) conjugate are added continuously to each micro-plate well and incubated. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human INHB. The concentration of Human INHB is calculated in the samples by comparing the OD of the samples to the standard curve according to Walker and Crowther [11]. Data were reviewed and transferred into computerized database using statistical software; the Statistical Package for the Social Sciences (SPSS). Descriptive statistics presented as frequencies (numbers), percentage (%), mean and standard deviation according to the variable type to compare two means of a variable, Student's t test for independent two groups

were applied in variables that followed the normal statistical distribution, in variables that did not follow the statistical normal distribution, the non-parametric "Mann-Whitney" test was applied. All statistical procedures and tests were performed under assumption of level of significance (P. value) of 0.05 or less to be significant difference or correlation.

### Ethical consideration

All candidates provided informed consent, consistent with the study protocol which was approved by the institutional ethics committee. The research was implemented according to the ideologies of the Helsinki Declaration.

## Results

The studied sample included 188 men with primary infertility due to non-obstructive azoospermia. 45.7% (86) were positive for testicular sperm extraction surgery and 54.3% (102) were negative for the same procedure as shown in figure (1).

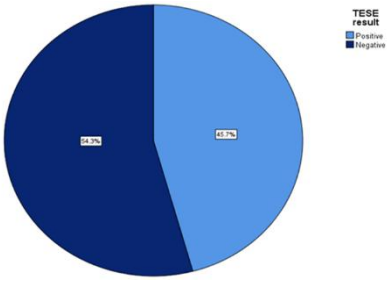



**Figure 1:** Pie chart represents TESE results in studied group.

As shown in Table 1, the mean age in the TESE-positive group was 35.2 ± 7.7 years, which is significantly higher than that of the TESE-negative group (31.7 ± 7.1 years). Mean of duration of infertility in the TESE positive was 9.1±6.3 years which is significantly more than that of the TESE negative 6.4±4.3 years. No significant statistical difference in BMI between both groups.

| Parameter | TESE result | | P₮ |
|---|---|---|---|
| | Positive (n=86) Mean±SD | Negative (n=102) Mean±SD | |
| Age (years) | 35.2±7.7 | 31.7±7.1 | 0.002* |
| Duration of infertility (years) | 9.1±6.3 | 6.4±4.3 | 0.001* |
| BMI (kg/m²) | 26.8±3.7 | 26.5±5.3 | 0.8 |

*Significant₮: student t-test TESE: Testicular sperm extraction

**Table 1:** Comparison of mean age, duration of infertility and BMI according to TESE results.

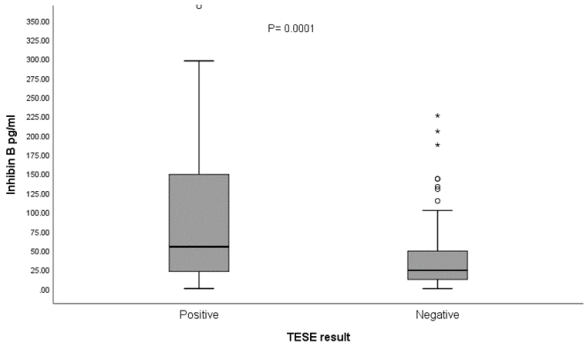
Regarding serum FSH, LH, Prolactin and testosterone, the median of their values compared according to TESE results in table (2). The values are represented as median and interquartile range. There is a significant statistical difference in FSH and LH level between both groups. while Prolactin and testosterone had no significant difference with TESE results.

The values of serum inhibin B are measured and compared for TESE positive and TESE negative groups. The median of INHB for TESE positive was (54.7pg/ml) which is significantly higher than median of TESE negative (24pg/ml) with P value=0.0001, figure (4.2) show that explanation.

| Hormone | TESE result | | P Ɱ |
|---|---|---|---|
| | Positive n=86 Median (IQR)# | Negative n= 102 Median (IQR) # | |
| FSH(mIU/ml) | 5.1 (11.4) | 20.1 (21.6) | 0.0001* |
| LH (mIU/ml) | 3.7 (2.8) | 7.2 (8.02) | 0.0001* |
| Prolactin (ng/ml) | 8.2 (5.4) | 9.3 (11.3) | 0.1 |
| Testosterone (ng/dl) | 355.4 (202.7) | 318.1 (393.02) | 0.4 |

*Significant, #IQR: interquartile range, Ɱ: Mann-Whitney test
FSH: follicle stimulating hormone
LH: luteinizing hormone

**Table 2:** Comparison of FSH, LH, Prolactin and Testosterone according to TESE results.




* High extreme values ◦ High potential outliers

**Figure 2**: Whisker boxplot comparing median inhibin B (pg/ml) according to TESE results plotted as positive and negative results.

Figure 3 displays the Receiver Operating Characteristic (ROC) curve, which illustrates the diagnostic performance of serum inhibin B by plotting the true positive rate (sensitivity) against the false positive rate (1 - specificity). The best cutoff point of serum INHB was 24 pg/ml give 70.9% sensitivity and 50% specificity.

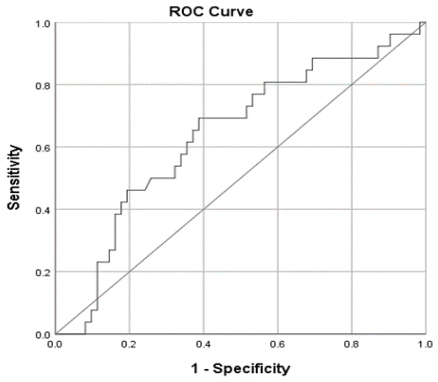



**Figure 3:** Receiver operating characteristic (ROC) curve of INHB.

| Area Under the Curve | | | | |
|---|---|---|---|---|
| Test Result Variable(s): Inhibin B pg/ml | | | | |
| Area | Std. Error | *p* value | 95% Confidence Interval | |
| | | | Lower Bound | Upper Bound |
| 0.639 | 0.065 | 0.041* | 0.511 | 0.766 |

**Table 3:** Detailed receiver operating characteristic (ROC) curve.

Table 4 explains the role of serum INHB as a predictor using a cutoff value of 24 pg/ml, with the following results: True Positives (TP) = 61 (70.9%); False Negatives (FN) = 25 (29.1%); False Positives (FP) = 51 (50%); and True Negatives (TN) = 51 (50%). So, according to the equation TP/(TP+FN) sensitivity of the test is 70.9%, and by the equation TN/(TN+FP) the specificity of the test is 50%, with diagnostic accuracy 59%. (TP: true positive; FN: false negative; FP: false positive; TN: true negative). The values are represented as frequencies (numbers) and percentage (%)

| Inhibin B Status | | TESE result | | Total |
|---|---|---|---|---|
| | | Positive NO. (%) | Negative NO. (%) | |
| Inhibin B (pg/ml) | Positive | 61 (70.9%) | 51 (50%) | 112 (59.6%) |
| | Negative | 25 (29.1%) | 51 (50%) | 76 (40.4%) |
| Total | | 86 (100%) | 102(100%) | 188 (100%) |

**Table 4:** Results of inhibin B test as predictor comparing to TESES results.

## Discussion

The physiological importance of INHB is to express the negative feedback effect on FSH secretion, and as the spermatogenesis present, Sertoli cell function still present and inhibin still secreted by these cells [12]. The optimal level of INHB for assessing male fertility has not yet been established [6].

The participants in this study were entered the testicular sperm retrieval surgery and the TESE was positive for 45.7%. In our study, the mean age of the TESE-positive group was higher than that of the TESE-negative group, suggesting a higher sperm retrieval rate in the older age group, similar to Kizilkan *et al.,* [13], this is may be due to their exposure to a second biopsy which may increase the chance of sperm retrieval or a documentation to the hypothesis which stated that older men with NOA may have secondary azoospermia due to environmental or acquired factors with better biopsy outcome [14]. Duration of infertility in the TESE positive was significantly more than that in TESE negative (the more duration the more prediction for sperm retrieval), this is supported by Saccà *et al.,* [15] who found a positive correlation of duration of infertility with TESE results. El-Haggar *et al.,* [16], Kizilkan *et al.,* [13] and Ghalwash *et al.,* [17] found no statistically significant association between period of infertility and sperm retrieval. Regarding BMI, this study showed no significant statistical association between BMI and TESE result. This is supported by study conducted by Li *et al.,* [18]. *Iwatsuki et al.,* [19].

Our results showed that median of FSH was significantly lower in TESE positive (5.1 mIU/ml) than TESE negative (20.1 mIU/ml). Colpi *et al.,*[20], Ghalayini *et al.,* [21] and Amer *et al.,*[22] found a significant association between low FSH levels and positive sperm retrieval (FSH significantly lower in positive than negative TESE). Regarding LH, the median was significantly lower in TESE positive (3.7 mIU/ml) than median in TESE negative (7.2mIU/ml). Saber-Khalaf *et al.,* [23] showed significant lower LH and FSH in positive sperm retrieval group. Regarding prolactin, the median had no statistically significant difference according to TESE results (p value= 0.1), this is supported by study conducted by Salehi *et al.,* [24] who found neither prolactin nor testosterone are significantly differ according to testicular sperm retrieval rate. Regarding testosterone, the median had no statistically significant difference according to TESE results (p value= 0.4), this is supported by study conducted by Althakafi *et al.,* [25] who found no significant difference in testosterone level in relation with testicular sperm retrieval results. Güneri *et al.,* [26] support all our results regarding FSH, LH, prolactin and testosterone. This study showed that serum INHB level in TESE positive was significantly higher than that in TESE negative group (54.7 pg/ml versus 24pg/ml). Similar results present in study conducted by Al-Bdairi *et al.,* [27] and Gamidov *et al.,* [28]. Barbotin *et al.*, [29] showed no significant difference in INHB levels based on TESE results in NOA, but their study was limited to patients with cryptorchidism. In our study, INHB with cut off value 24 pg/ml had 70.9% (nearly 71%) sensitivity and 50% specificity, this was near to results obtained by Huang *et al.,* [30]. According to the above-mentioned data serum INHB is a supportive marker for the diagnosis with accepted level of sensitivity and specificity for sperm retrieval rate and has no accurate or conclusive predictive role for sperm recovery in male with non-obstructive azoospermia. Our result is supported by Tunc *et al.,* [31] who showed that INHB is not predictive for sperm retrieval with sensitivity 90% and specificity only 14%, Radkhah *et al.,* [32].

It is important to note that the sensitivity and specificity of serum INHB may increase when comparing patients with NOA to those with obstructive azoospermia (OA) or to normal controls (for male fertility assessment), as conducted by Kong *et al.,* [33] who showed that 92% sensitivity and 88% specificity with INHB cut off 45.9 pg/ml when comparing obstructive and non-obstructive azoospermia, but this is not our aim in this study. In contrast, some studies such as Ballescá *et al.,* [34] made INHB as predictor for presence of sperm by TESE in NOA cases, with sensitivity 90%, specificity 100% (cut off >40 pg/ml). A systematic review done by Deebel *et al.,* [35]

highlighted on role of INHB in predicting sperm retrieval in NOA with Klinefelter syndrome, showed that INHB cannot predict sperm retrieval.

Most of studies in the literature took both INHB and FSH as markers to either predict or not predict spermatogenesis of NOA patients. Al-Bdairi *et al.,* [26] concluded that both INHB and FSH are reliable noninvasive markers to predict the outcome of sperm retrieval. Several researchers such as Deng *et al.,* [36] utilize both INHB and AMH as tool for sperm prediction as they are both testicular hormones and of Sertoli cell origin, the mentioned study found INHB/AMH ratio give promising results about sperm retrieval. Besides these conflicting data and the controversies about the "direct marker" role of serum Inhibin B in determination of spermatogenesis, it does not seem to give a clue about the presence of sperm before TESE, as focal spermatogenic activity is very little to have stimulatory effect on Sertoli cells to produce INHB. So INHB measurement can be used to reflect the function of testicular tissue in general, but cannot predict exactly the presence of sperm.

We suggest that serum INHB can be used as a supportive test for counseling patients with NOA, rather than as a definitive marker for the presence or absence of sperm prior to performing TESE. Testicular biopsy and histopathology remain the cornerstone and gold standardmethod in diagnosing the presence or absence of focal spermatogenesis in NOA patients. Serum levels of Inhibin B hormone is significantly higher in TESE positive than TESE negative group. With a cut off 24pg/ml the sensitivity is 70.9% and specificity 50%, so Inhibin B is not far enough sensitive and specific to predict sperm retrieval and should not be used in clinical practice as marker to predict sperm retrieval.

## Competing Interests

The authors declared that there were no conflictsof interest.

## Author Contributions

AAA: Research idea, study design and manuscript, HA: Data collection and Data analysis, RHA: Interpreted the results, WSM: Logistic support. All authors discussed the findings and provided feedback on the text.

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