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A PROSPECTIVE STUDY ON TESTICULAR MAPPING AND ITS CO-RELATION WITH SERUM HORMONAL LEVELS.

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Abstract

Background

Azoospermia, the complete absence of sperm in the ejaculate, is a major cause of male infertility, affecting 1% of men globally. It is categorized into obstructive azoospermia (OA) and non-obstructive azoospermia (NOA), with NOA being more prevalent and presenting challenges for sperm retrieval. Despite advances in techniques like intracytoplasmic sperm injection (ICSI), reliable markers for successful sperm retrieval in NOA remain unclear.

Aims

This study aims to evaluate histopathological abnormalities in azoospermic males and assess the efficacy of testicular mapping for sperm retrieval. It also focuses on the diagnostic value of serum inhibin B and other hormones in distinguishing OA from NOA.

Methods

This was a prospective study involving 54 male patients diagnosed with azoospermia, assessed through a compound testicular mapping procedure with 14 microbiomes. Hormonal assessments such as follicular stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and inhibin B were conducted before and after the biopsy. Histopathological analysis was focused on confirming azoospermia, differentiating OA from NOA, and identifying focal spermatogenesis. Statistical analysis correlates hormone levels with biopsy findings to evaluate diagnostic outcomes.

Results

The study analyzed 54 cases of azoospermia in men aged 20-39 years, with non-obstructive azoospermia (NOA) being more prevalent (74.1%) than Obstructive Azoospermia (OA) (25.9%). NOA cases were further classified into hypospermatogenesis, maturation arrest, and Sertoli cell-only syndrome. Hormonal analysis revealed significant differences in serum LH, FSH, testosterone, and inhibin B levels across azoospermia subtypes, with serum inhibin B showing the highest sensitivity and specificity for differentiating OA and NOA. The findings underscore hormonal variability and the potential of inhibin B as a clinical marker for azoospermia classification.

Conclusion

The study highlights significant hormonal differences between obstructive and nonobstructive azoospermia subtypes, with serum Inhibin B emerging as the most reliable marker for differentiation.

Keywords: *Azoospermia, Nonobstructive Azoospermia, Obstructive Azoospermia, Hormonal Markers.*
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Introduction

Globally, approximately 15% of couples are affected by infertility, with azoospermia—a condition that is distinguished by the absence of sperm in the ejaculate—playing a substantial role. Worldwide, azoospermia affects about 1% of men and 10–20% of infertile men. Nonobstructive azoospermia (NOA) is most commonly caused by testicular failure, while obstructive azoospermia occurs when the reproductive system becomes blocked. The specific hurdles presented by NOA, which accounts for the majority of azoospermic cases, stem from the patchy or "focal" form of spermatogenesis, which makes sperm retrieval a challenging process [1-4]. Because clinical indicators such as testicular size, hormone levels, and biopsy patterns do not always consistently show the existence of mature sperm, the successful retrieval of viable sperm in NOA patients is complicated and unpredictable, even with advanced assisted reproductive techniques like

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intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF) [5-7].

Recent advancements in sperm retrieval techniques, such as fine needle aspiration (FNA) mapping, aim to improve success rates by systematically sampling multiple regions within the testicle [8,9]. FNA mapping enables targeted sperm retrieval by identifying testicular regions that are more likely to contain mature sperm [10]. Despite these advances, a significant gap exists in understanding the histopathological factors and hormone levels that correlate with successful sperm retrieval [11,12]. Hormones such as follicle-stimulating hormone (FSH) and inhibin B (INHB) are closely linked to spermatogenesis, yet their exact diagnostic value in evaluating azoospermic men remains unclear [13-15]. By examining testicular biopsies and serum hormone levels, *this study seeks to clarify the relationship between these factors and sperm presence in azoospermic men, potentially enhancing retrieval strategies and patient outcomes.*

Aim of the study

The main objective of this study is to evaluate the histopathological abnormalities in azoospermic males, exploring the efficacy of mapping and multiple microbiopsies to guide sperm retrieval. Additionally, it seeks to assess hormonal abnormalities, focusing on serum inhibin B and other hormones, to understand their diagnostic value in classifying azoospermia as obstructive or nonobstructive.

Methods

Study Setting

This study was conducted at the Department of Urology and Renal Transplantation and the Department of Pathology, SCB Medical College and Hospital, from December 2019 to December 2021.

Study Population

The study included a minimum of 54 male patients with infertility, specifically those attending the Andrology Outpatient Department (OPD) who have been diagnosed with azoospermia on semen analysis.

Inclusion Criteria

The study included male patients in the age group of 20-39 years who were experiencing primary infertility, as confirmed by azoospermia on semen analysis. Azoospermia must be documented by WHO semen analysis criteria. Only those patients meeting these criteria were eligible for participation to ensure a consistent study population with similar infertility profiles.

Exclusion Criteria

Individuals with specific medical conditions were not eligible, including those with testicular malignancies, which may interfere with biopsy findings, and those with ejaculatory disorders such as retrograde ejaculation, which complicates sperm retrieval. Patients with intractable bleeding disorders are also excluded due to the increased risk of complications during biopsy procedures. These exclusion criteria help maintain participant safety and the reliability of study results.

Study Method

This study employed a compound testicular mapping procedure to assess azoospermic men. Two types of mapping approaches are typically used: simple maps, which involve four or fewer biopsy sites for diagnostic purposes, and compound maps, which entail more extensive sampling with more than four sites and are primarily used in cases of nonobstructive azoospermia (NOA). In our study, each testis underwent a compound mapping procedure with 14 microbiopsies. This approach intends to strike a balance between patient comfort as well as the need for comprehensive tissue sampling, thereby maximizing the likelihood of locating viable sperm in focal areas.

Study Design

The research followed a prospective study design. This approach enables longitudinal assessment of biopsy and mapping outcomes while allowing careful tracking of histopathological and hormonal correlations in azoospermic men.

Data Collection

Data collection was commenced once ethical clearance was granted, and written informed consent was obtained from all participants. Clinical and diagnostic information—including semen analysis (per WHO standards), trans-scrotal ultrasound findings for testicular volume and echogenicity, and hormonal assessments for FSH, LH, estradiol, testosterone, and inhibin B—was recorded before and following the biopsy procedure. These data provided a comprehensive baseline and post-procedure profile for each participant.

Methodology

Each testicular sample underwent 14 microbiopsies, fixed in neutral-buffered formalin for 18 to 24 hours and subsequently embedded in paraffin. Sections were then stained with hematoxylin and eosin (H&E) for histopathological examination. The analysis was focused on several key criteria, including confirmation of azoospermia, differentiation between obstructive and non-obstructive azoospermia, correlation between hormone levels and biopsy findings, and identification of focal spermatogenesis in NOA cases.

Statistical Analysis

Statistical analysis was conducted to explore and quantify the relationships between histopathological findings, hormonal profiles, and the likelihood of locating mature sperm, providing valuable insights into the diagnostic and prognostic aspects of azoospermia. The data was either presented as n or mean±SD. The Kruskal-Wallis test was used to obtain the p-value.

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Results

The study examined 54 cases of azoospermia confirmed by seminal analysis in men aged 20 to 39 years, with the largest group in the 30-34 year age range. Histopathologically, cases were categorized into two main types: Nonobstructive Azoospermia (NOA) and Obstructive Azoospermia (OA). NOA was more prevalent, representing 74.1% of cases, while OA accounted for 25.9%. Further examination of NOA cases revealed three subtypes: hypospermatogenesis (33.3%), maturation arrest (16.7%), and Sertoli cell-only syndrome (24.1%). The findings highlight that a majority of azoospermia cases were nonobstructive, with hypospermatogenesis being the most common subtype among NOA cases (Table 1).

Table 1: Characteristics of the patients enrolled in this study:

Category	Subcategory	Number of Cases	Percentage (%)
Age Group (Years)	**20-24**	1	1.9
	25-29	15	27.8
	30-34	23	42.6
	35-39	15	27.8
HP Diagnosis	**Nonobstructive Azoospermia**	40	74.1
	Obstructive Azoospermia	14	25.9
Testicular Biopsy Findings (NOA)	**Hypospermatogenesis**	18	33.3
	Maturation Arrest	9	16.7
	Sertoli Cell Only Syndrome	13	24.1
	Obstructive Azoospermia	14	25.9

The hormonal levels across different histopathological diagnoses of azoospermia showed significant variation. Serum LH, FSH, testosterone, and inhibin B levels were significantly different among the subtypes of NOA and OA, with Kruskal-Wallis test p-values < 0.05. Among NOA cases, Sertoli cell-only syndrome exhibited the highest serum LH and testosterone levels, while hypospermatogenesis had the lowest. Serum inhibin B levels were also lowest in Sertoli cell-only syndrome. In contrast, OA cases had significantly lower mean values for LH and testosterone but higher levels of inhibin B compared to NOA cases. For serum prolactin, no significant differences were observed between OA and NOA (p-value = 0.122), indicating that prolactin levels were consistent across the groups. These findings emphasize hormonal variability in azoospermia subtypes, particularly in LH, testosterone, and inhibin B, which could aid in understanding the underlying mechanisms of infertility (Table 2) (Figure 1).

Table 2: Hormonal Correlation by Histopathological Diagnosis in Azoospermia Cases

HP Diagnosis	Serum LH in µg/ml (Mean ± S.d)	Serum FSH µg/ml (Mean ± S.d)	Serum Testosterone in ng/dl (Mean ± S.d)	Serum Prolactin in ng/ml (Mean ± S.d)	Serum Inhibin B in pg/ml (Mean ± S.d)
Nonobstructive Azoospermia (Hypospermatogenesis)	10.88 ± 2.90	26.75 ± 2.96	364.94 ± 215.13	13.14 ± 6.89	41.05 ± 21.33
Nonobstructive Azoospermia (Maturation Arrest)	11.20 ± 2.54	30.59 ± 6.37	424.00 ± 169. 44	14.10 ± 3.41	64.91 ± 18.32
Nonobstructive Azoospermia (Sertoli Cell Only Syndrome)	15.58 ± 2.02	25.32 ± 7.59	681.54 ± 32.08	10.04 ± 0.71	7.64 ± 3.30
Obstructive Azoospermia	4.24 ± 2.64	10.78 ± 9.57	739.29 ± 65.83	11.34 ± 4.40	187.15 ± 24.17
Kruskal-Wallis Test Significance	p-value: 0.000	p-value: 0.000	p-value: 0.000	p-value: 0.122	p-value: 0.000

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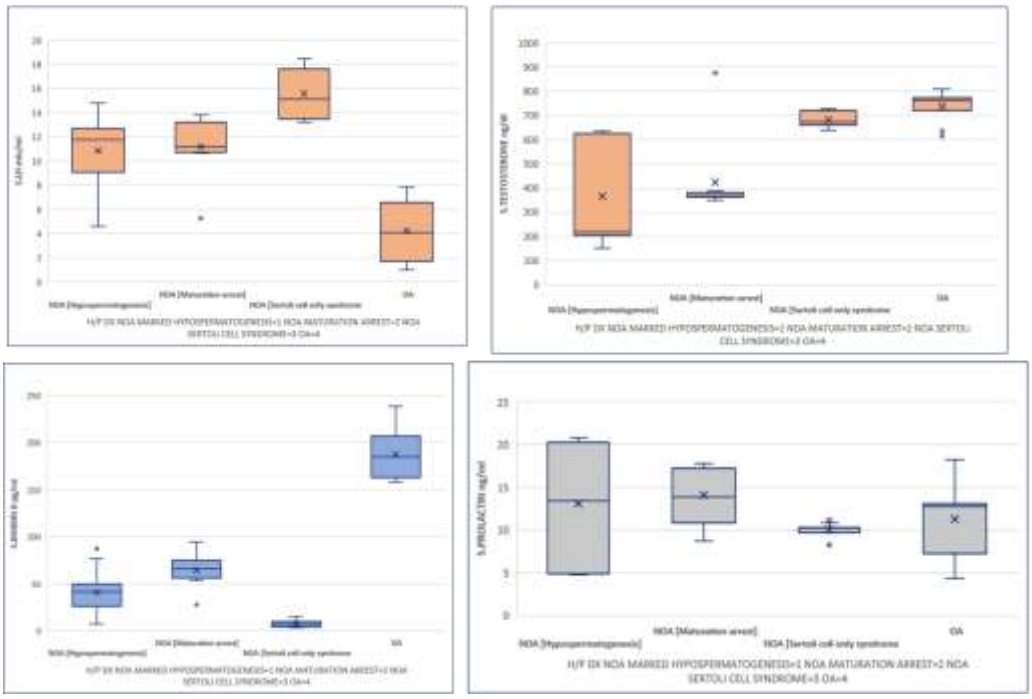

Figure 1: Independent- Sample Kruskal-Wallis Test.

The pairwise comparison test was applied to assess the significance of various hormones in differentiating between different types of azoospermia. For serum LH, significant differences were found between OA and all NOA subtypes, particularly with Sertoli cell-only syndrome ($p = 0.000$). Similarly, for serum FSH, OA could be significantly differentiated from all NOA subtypes, though no significant distinctions were found between the NOA subtypes themselves. In serum testosterone, significant differentiation was noted between OA and both NOA hypospermatogenesis and NOA maturation arrest, as well as between NOA hypospermatogenesis and Sertoli cell-only syndrome. Lastly, for serum inhibin B, OA cases had significantly different levels from both NOA hypospermatogenesis and Sertoli cell-only syndrome. However, NOA maturation arrest did not show significant differences from the other subtypes. Overall, these results indicate that hormones like LH, FSH, testosterone, and inhibin B are effective in distinguishing OA from various NOA subtypes, with serum prolactin showing no significant differences (Table 3).

Table 3: Pairwise Comparison for Hormonal Differentiation Between Azoospermia Types

Hormone	Sample 1 - Sample 2	Significance
Serum LH	OA - NOA (Hypospermatogenesis)	0.006
	OA - NOA (Maturation Arrest)	0.024
	OA - NOA (Sertoli Cell Only Syndrome)	0.000
	NOA (Hypospermatogenesis) - NOA (Maturation Arrest)	1.000
	NOA (Hypospermatogenesis) - NOA (Sertoli Cell Only Syndrome)	0.006
	NOA (Maturation Arrest) - NOA (Sertoli Cell Only Syndrome)	0.051
Serum FSH	OA - NOA (Hypospermatogenesis)	0.002
	OA - NOA (Maturation Arrest)	0.000
	OA - NOA (Sertoli Cell Only Syndrome)	0.026
	NOA (Hypospermatogenesis) - NOA (Maturation Arrest)	0.316
	NOA (Hypospermatogenesis) - NOA (Sertoli Cell Only Syndrome)	1.000
	NOA (Maturation Arrest) - NOA (Sertoli Cell Only Syndrome)	0.148
Serum Testosterone	OA - NOA (Hypospermatogenesis)	0.000
	OA - NOA (Maturation Arrest)	0.004
	OA - NOA (Sertoli Cell Only Syndrome)	1.000
	NOA (Hypospermatogenesis) - NOA (Maturation Arrest)	1.000
	NOA (Hypospermatogenesis) - NOA (Sertoli Cell Only Syndrome)	0.000

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	NOA (Maturation Arrest) - NOA (Sertoli Cell Only Syndrome)	0.104
Serum Inhibin B	OA - NOA (Hypospermatogenesis)	0.000
	OA - NOA (Maturation Arrest)	0.157
	OA - NOA (Sertoli Cell Only Syndrome)	0.000
	NOA (Hypospermatogenesis) - NOA (Maturation Arrest)	1.000
	NOA (Hypospermatogenesis) - NOA (Sertoli Cell Only Syndrome)	0.029
	NOA (Maturation Arrest) - NOA (Sertoli Cell Only Syndrome)	0.002

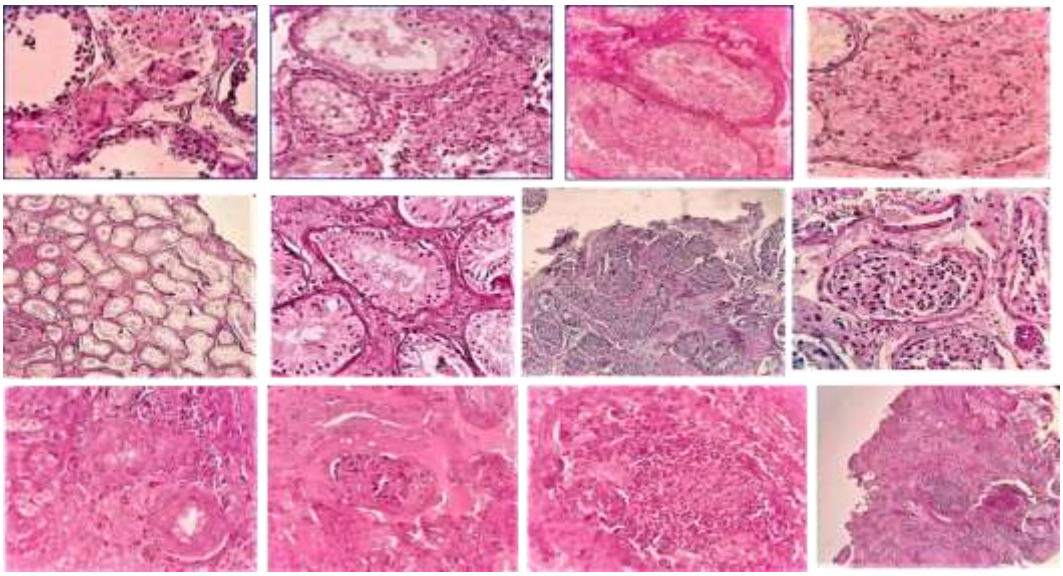

Figure 2: Histological Features of Various Types of Azoospermia

Marked Hypospermatogenesis (H & E X400), Hypospermatogenesis with Tubular Sclerosis (H & E X400), Maturation Arrest (H & E X400), Leydig Cell Aggregates (H & E X400), Sertoli Cell Only Syndrome (H & E X100), Sertoli Cell Only Syndrome (H & E X400), Obstructive Azoospermia, Disorganised Pattern (H & E X100), Obstructive Azoospermia, Disorganised Pattern (H & E X400), Tubular Hyalinisation (H & E X400), Complete Tubular Hyalinisation with Few Spermatocytes (H & E X400), Granuloma (H & E X400), Granuloma with Tubular Hyalinisation (H & E X100) (in clockwise direction).

The ROC analysis demonstrated that serum Inhibin B had the highest sensitivity (100%) and specificity (100%), with an area under the curve (AUC) of 1.000, indicating its optimal performance as a differentiating marker between OA and NOA. Following Inhibin B, serum testosterone showed high sensitivity (97.5%) but lower specificity (79%), with an AUC of 0.879, suggesting its moderate efficacy as a clinical marker. Serum FSH exhibited good sensitivity (95%) but lower specificity (85%), with an AUC of 0.907, while serum LH had the lowest sensitivity (87.5%) but the highest specificity (100%), with an AUC of 0.959. Overall, serum Inhibin B proved to be the most reliable marker for differentiating between OA and NOA, while serum testosterone and serum FSH also performed well, though with varying specificity (Table 4).

Table 4: ROC Curve Findings for Various Hormones

Hormone	Sensitivity	Specificity	Area Under Curve
Serum LH	87.5%	100%	0.959
Serum FSH	95%	85%	0.907
Serum Testosterone	97.5%	79%	0.879
Serum Inhibin B	100%	100%	1.000

Discussion

In this study, a total of 54 cases of azoospermia were analyzed within the 20 to 39-year age group. Among these, the highest number of cases (42.5%, n=23) was found in the 30–34-year age range. However, no significant relationship was observed between the age group and the type of azoospermia, aligning with findings from Wen-Hao Tang et al. (2018), who also reported no correlation between age and azoospermia type. This indicates that age may not be a crucial factor in determining the type of azoospermia, as corroborated by earlier studies in the field [16].

Azoospermia was categorized into OA and NOA types in our study. Non-obstructive azoospermia (74.1%) was found to be more prevalent than obstructive azoospermia (25.9%), a trend that is consistent with findings from Danilo L. Andrade et al. (2021) [17]. Among the subtypes of NOA, hypospermatogenesis (33.3%) was the most common, followed by Sertoli cell-only syndrome (24.1%) and maturation arrest (16.7%). These findings echo those of Abdullah et al. (2011), who similarly identified hypospermatogenesis as the predominant histological pattern in NOA cases.

In terms of histopathological patterns, four distinct types were observed: hypospermatogenesis, maturation arrest, Sertoli cell-only syndrome, and obstructive azoospermia. The histological characteristics of each subtype included variations in seminiferous tubular diameters, fibrosis of the tubular basement membrane, and alterations in spermatogenesis. Hypospermatogenesis presented a reduction in spermatogenesis and occasional spermatids, while maturation arrest showed seminiferous tubules with only primary spermatocytes. Sertoli cell-only syndrome, on the other hand, exhibited tubules with only Sertoli cells and no germ cells. Obstructive azoospermia cases were characterized by tubular dilatation and a full range of spermatogenesis with disordered germ cell arrangements. These observations align with those of I.-S. Huang et al. (2018) highlighted the importance of distinguishing between obstructive and non-obstructive azoospermia in clinical practice [18].

The study also explored the correlation between hormone levels and the different types of azoospermia. Hormonal assessment, particularly for LH, FSH, testosterone, and inhibin B, provided valuable insights into distinguishing between obstructive and non-obstructive azoospermia. Our results analyzed using the Kruskal-Wallis test and receiver operating characteristic (ROC) curve, demonstrated significant differences in the serum levels of LH, FSH, and testosterone across the different histopathological diagnoses. Notably, serum LH levels were significantly lower in obstructive azoospermia cases compared to non-obstructive cases, with a cutoff value of 8.76 mIU/ml showing high sensitivity (87.5%) and specificity (100%). This finding supports the work of Xiangbin Kong et al. (2021) and I.-S. Huang et al. (2018) also reported lower LH levels in obstructive azoospermia [19].

The serum FSH cutoff value for distinguishing between azoospermia types was found to be 18.44 mIU/ml, with high sensitivity (95%) and specificity (85%). This is consistent with the findings of Ettore Caroppo et al. (2017), who observed higher serum FSH levels in cases of NOA compared to obstructive azoospermia. Similarly, serum testosterone levels were significantly higher in obstructive azoospermia cases, with a cutoff value of 739 ng/dl demonstrating 97.5% sensitivity and 79% specificity. These results align with studies by Huang et al. (2018) and Kong et al. (2021), which also reported higher testosterone levels in OA compared to NOA cases [18,19].

The cutoff value of 126.46 pg/ml was determined to be highly discriminative, indicating that serum inhibin B is a marker with 100% sensitivity and specificity. This is in agreement with the results of Sigrid von Eckardstein et al. (1999) and Xiangbin Kong et al. (2021), who detected lower levels of inhibin B in NOA cases and higher levels in obstructive azoospermia. Additionally, the significance of inhibin B as a diagnostic tool for distinguishing between azoospermia types is underscored by its high specificity and sensitivity in our study [19,20].

Intriguingly, there was no discernible distinction in serum prolactin levels between obstructive and non-obstructive azoospermia, as both groups exhibited comparable levels. This finding is in agreement with the findings of Xiangbin Kong et al. (2021), who also discovered that there was no significant correlation between prolactin levels and the form of azoospermia. Therefore, serum prolactin may not be a dependable indicator for distinguishing between OA and NOA [19].

Overall, our results were consistent with the notion that a combination of blood hormone levels, including those of LH, FSH, testosterone, and inhibin B, can serve as useful diagnostic indicators for distinguishing between obstructive and non-obstructive azoospermia. In cases where testicular biopsy may not be immediately feasible, hormonal assessment is particularly critical in the clinical management of azoospermia, as these findings are consistent with prior research.

Conclusion

This study highlights the key clinical and hormonal differences between obstructive and non-obstructive azoospermia (OA and NOA), emphasizing that NOA is more prevalent, with hypospermatogenesis being the most common subtype. The analysis of serum hormone levels revealed distinct patterns for each type, with serum LH, FSH, testosterone, and inhibin B providing valuable biomarkers for differentiating between OA and NOA, as well as for distinguishing between subtypes of NOA. Specifically, inhibin B was identified as a promising biomarker, either alone or in combination with other hormones, to aid in diagnosis. Although testicular biopsy remains an adjunct for confirming spermatogenesis, non-invasive serum hormone assessments offer a more convenient and reliable alternative for clinical practice, reducing the risks associated with invasive procedures. Overall, hormonal panels proved to be essential in the management of azoospermia, facilitating accurate diagnosis and guiding treatment decisions.

Data Availability

Data is available upon request.

Author contributions

All authors contributed to the design of the research. PA and KTP collected and analyzed the data. VN, SKR, and DH wrote the manuscript. All authors read, edited, and approved the paper.

List of Abbreviations

AUC- Area under the curve
FSH- Follicle Stimulating Hormone
LH- Luteinizing Hormone
OA- Obstructive azoospermia
NOA- Non-obstructive azoospermia
ICSI- Intracytoplasmic sperm injection
IVF- In vitro Fertilisation
FNA- Fine needle aspiration
INHB- Inhibin B
OPD- Outpatient Department

Conflict of Interest

There was no conflict of interest regarding the study.

Source of Funding

No funding was received.

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