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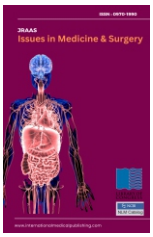

Research Article

Section: Pathology

Needling Infertility: Diagnostic Utility of Fine Needle Aspiration Cytology (FNAC) of Testes in the Evaluation of Male Infertility

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HIGHLIGHTS

- FNAC enables minimally invasive diagnosis
- Differentiates OA versus NOA
- Correlates cytology with hormonal profile
- MA most common finding
- Cost-effective in resource settings

Key Words:
Testicular FNAC
Male infertility
Azoospermia
Sertoli Cell-Only Syndrome
Maturation Arrest
Hypospermatogenesis
Testicular Cytology
Non-obstructive Azoospermia

ABSTRACT

Introduction: Male infertility affects nearly 15% of reproductive-age couples, with male factors contributing to about 50% of cases. Azoospermia, seen in 10–15% of infertile men, requires differentiation into obstructive and non-obstructive types. While a testicular biopsy is definitive, it is invasive. Fine Needle Aspiration Cytology (FNAC) offers a minimally invasive, cost-effective alternative for assessing spermatogenesis. **Aim & Objectives:** This study aimed to evaluate the diagnostic role of testicular FNAC in infertile males by classifying cytological patterns, correlating findings with semen analysis and hormonal profiles, and distinguishing obstructive from non-obstructive infertility. **Material & Methods:** A 12-month prospective study was conducted at GSVM Medical College and LLR Hospitals, Kanpur, including 140 infertile males with azoospermia or severe oligospermia (<5 million/mL). All underwent semen analysis (WHO criteria), hormonal assays (FSH, LH, testosterone, prolactin), and scrotal ultrasonography. FNAC was performed using a 23–25G needle. Smears were stained with May-Grünwald-Giemsa, Papanicolaou, and H&E, and categorized into five groups: normal spermatogenesis, hypospermatogenesis, maturation arrest, Sertoli cell-only syndrome, and testicular atrophy. **Results:** Among the 140 patients evaluated, normal spermatogenesis was observed in 29.3% of cases, all presenting with azoospermia and normal FSH levels, suggesting obstructive azoospermia. Hypospermatogenesis was also identified in 29.3% of patients and was commonly associated with borderline elevated FSH levels and oligospermia. Maturation arrest was the most frequent finding, seen in 35% of cases, predominantly in early stages, and correlated with elevated FSH and low testosterone levels. Sertoli cell-only syndrome was diagnosed with 0.7% of patients, all of whom had markedly elevated FSH levels and azoospermia. Testicular atrophy was observed in 5% of cases, indicating advanced testicular failure. **Conclusion:** FNAC is a safe, effective first-line diagnostic tool for evaluating male infertility, enabling accurate classification and guiding appropriate management, especially in resource-limited settings

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INTRODUCTION

Infertility remains a major public health issue in India, affecting approximately 10–15% of couples. In nearly half of these cases, male factors play a significant or contributory role [1]. In India, estimates are often in the range of 40-50% for male factor involvement. A scoping review in the Asian Pacific Journal of Reproduction noted that Indian studies show male factor infertility broadly in ~40% of infertility cases [2]. Among the causes of male infertility, azoospermia, which is defined as the complete absence of sperm in the ejaculate, is particularly challenging. It occurs in about 1% of the general male population and up to 15% of infertile men [3]. Azoospermia can be broadly classified into two categories: Obstructive Azoospermia (OA), where sperm production is normal but transport is impeded due to anatomical or acquired blockage, and Non-Obstructive Azoospermia (NOA), characterized by intrinsic testicular failure and impaired spermatogenesis [4].

In the study **"Male infertility in India: Demographics, aetiology and outcomes of standard clinical practice"** with 447 male patients, of whom 40% had azoospermia, 21.1% of the total patients had obstructive azoospermia. Since only 40% had azoospermia, this corresponds to roughly about 52–53% of azoospermic men being a case of the obstructive category.

Reliable large-scale, peer-reviewed data specific to India are limited, but studies suggest:

- NOA may account for 50–60% of azoospermic cases in most Indian tertiary care settings.
- OA ranges from 40–50% based on studies like: Dutta et al. (2021)- [International Journal of Reproduction, Contraception, Obstetrics and Gynecology (IJRCOG)]: NO A seen in 53.7% of azoospermic men. Saha et al. (2020): Similar NOA dominance in the Eastern Indian cohort.

Thus, even in India, the NOA: OA ratio is approximately 3:2 or 5:4, depending on region and diagnostic accuracy.

Accurately distinguishing OA from NOA is crucial for clinical decision-making. While OA can sometimes be managed with reconstructive surgery, NOA typically necessitates more complex approaches such as testicular sperm extraction (TESE) followed by intracytoplasmic sperm injection (ICSI) [5]. This classification directly impacts prognosis, treatment cost, and the likelihood of achieving biological parenthood. Therefore, early and accurate diagnosis of the underlying azoospermia subtype is a cornerstone in the management of male infertility.

Traditionally, open testicular biopsy has served as the gold standard for evaluating spermatogenesis. Histological analysis of the seminiferous tubules provides detailed insights into

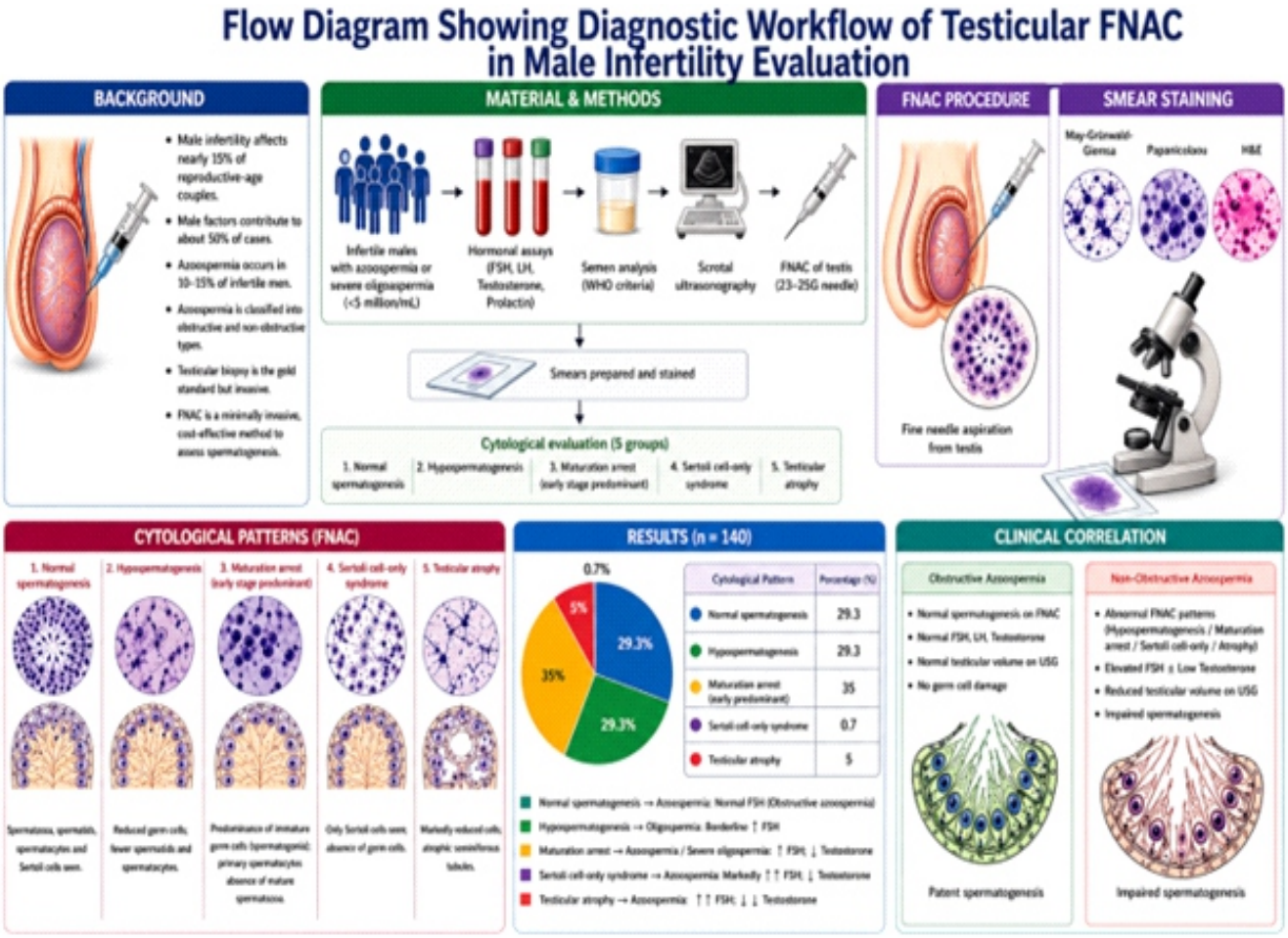

Figure 1: Flow Diagram Showing Diagnostic Workflow of Testicular FNAC in Male Infertility Evaluation. ***Adopted from Biorender.com***

testicular pathology, including patterns such as hypospermatogenesis, maturation arrest, Sertoli-cell-only syndrome (SCOS), and testicular atrophy [6]. However, the invasive nature of this procedure, its higher cost, requirement for operating room setup, potential complications (e.g., bleeding, pain, scarring), and patient apprehension make it less suitable, especially in resource-constrained settings like many regions of India [7]. Moreover, open biopsy is not ideal for repeat assessments due to its associated morbidity and limited patient acceptability. To overcome these limitations, Fine Needle Aspiration Cytology (FNAC) has gained traction as a less invasive, cost-effective, and efficient alternative to testicular biopsy [8].

FNAC involves aspirating testicular cells using a fine-gauge needle (typically 22–24G) under local anesthesia, followed by cytological examination of the sample. The testicular parenchyma is typically sampled from multiple locations to ensure representativeness. The aspirated material is then smeared, fixed, stained, and analyzed under light microscopy to assess the presence and stages of spermatogenic cells, sertoli cells, and other features indicative of the underlying testicular function [9].

Numerous Indian studies have validated the diagnostic accuracy of FNAC. In a prospective study by Kurien et al. involving 57 infertile men, a 91.9% concordance was reported between FNAC and biopsy findings [11]. Similarly, Sridevi and Karkuzhali reported an overall diagnostic accuracy of 86% in a 36 patient study, with especially high accuracy in diagnosing SCOS and maturation arrest [12]. A recent study by Mohanty et al. from Odisha (2025) demonstrated 97.9% agreement between FNAC and biopsy in 48 patients, further supporting FNAC as a reliable method for testicular evaluation in azoospermic men [13]. In yet another study from Madhya Pradesh involving 119 patients, FNAC showed a diagnostic yield of over 95%, with only 4.2% of samples deemed inadequate [14].

From a global perspective, the role of testicular FNAC has also been explored extensively. In Western settings, where ART services are advanced, FNAC has been used not only for diagnosis but also for sperm retrieval, especially in NOA patients. Studies from Europe and the Middle East report diagnostic accuracy rates of 84–97% for FNAC when compared with biopsy [15]. In Japan, testicular FNAC has been incorporated into clinical guidelines for initial male infertility workup, especially when ART is considered [16].

One of the most compelling utilities of FNAC lies in its potential to predict ART outcomes. Several studies have correlated FNAC findings with successful sperm retrieval via TESE. For example, the presence of late spermatids or spermatozoa in FNAC samples is strongly associated with higher success rates in sperm retrieval and, consequently, higher ICSI success rates [17]. Furthermore, FNAC can guide the selection of patients for micro-TESE, a technique often used in NOA patients to harvest sperm directly from focal areas of spermatogenesis. By atrophy, FNAC improves resource utilization and patient outcomes.

In addition to the biological and technical aspects, it is important to consider the ethical and psychosocial implications of male infertility and its diagnosis in India. Infertility, particularly male infertility, is often surrounded by stigma, shame, and social pressure. Many couples avoid seeking timely diagnosis due to cultural barriers, fear of blame, or the misconception that infertility is primarily a female issue [18]. In such contexts, less invasive procedures like FNAC are more likely to be accepted by patients, thereby promoting earlier diagnosis and appropriate treatment. Moreover, testicular biopsy-often associated with “loss of manhood” in some rural communities-can deter patients from pursuing care. Offering FNAC as a minimally invasive, outpatient-based diagnostic tool reduces this barrier and supports equitable access to infertility care (**Figure 1**) [19].

MATERIALS & METHODS

This prospective, observational, and comparative study was conducted in the Departments of Pathology and Urology at GSVM Medical College and Associated Hospitals, Kanpur, over 12 months (January–December 2025) and included male patients presenting with infertility and referred for evaluation of azoospermia. Patients aged 20–50 years diagnosed with azoospermia or severe oligospermia (<5 million/mL) on at least two semen analyses as per WHO criteria and willing to provide informed consent were included, while those with prior testicular surgery or trauma, suspected malignancy, systemic illnesses affecting testicular function, or bleeding disorders were excluded. A total of 140 patients were enrolled, and both testes were clinically and ultrasonographically evaluated; unilateral FNAC was performed in cases with symmetrical testes, while bilateral sampling was done in asymmetrical cases. FNAC was carried out under aseptic precautions and local anesthesia (2% lignocaine) using a 22–24-gauge needle, and smears were prepared, air-dried, and stained with May Grünwald Giemsa and Hematoxylin and Eosin stains. Cytological evaluation included assessment of at least 100 cells per slide, focusing on spermatogenic cells and classification into normal spermatogenesis, hypospermatogenesis, maturation arrest, Sertoli cell-only syndrome, and tubular atrophy. Clinical, demographic, semen analysis, hormonal (FSH, LH, testosterone), and ultrasonographic data were recorded for all patients. Statistical analysis was performed using SPSS version 27, with descriptive statistics and inferential tests such as Chi-square, Student's t-test, and Pearson correlation applied, considering $p < 0.05$ as statistically significant, and Kappa statistics used to assess agreement between FNAC and biopsy findings.

RESULT

Table 1 summarizes the semen analysis and hormonal profile findings of all study participants. It presents the distribution of

patients based on semen characteristics and serum hormone levels, including luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone. All participants in the present study were diagnosed with azoospermia, as confirmed by semen analysis (Table 1). Assessment of serum gonadotropin levels (LH and FSH) revealed that all 140 patients (100%) had normal LH/FSH concentrations, indicating that most cases were likely of non-hypergonadotropic origin. However, evaluation of serum testosterone levels demonstrated that 99 patients (70.7%) had subnormal levels, while 41 patients (29.3%) maintained normal testosterone levels. These findings suggest that although gonadotropin levels remained within the physiological range, a significant proportion of azoospermic men exhibited compromised Leydig cell function, as reflected by subnormal testosterone concentrations. This hormonal profile supports the presence of testicular dysfunction as a contributing factor to infertility in the study population. **Table 2** presents the distribution of cytological diagnoses obtained from fine-needle aspiration cytology (FNAC) of testicular aspirates, highlighting the frequency and percentage of each cytological pattern. Fine-needle aspiration cytology (FNAC) of testicular aspirates revealed a range of urogenital findings among the 140 azoospermic patients (Table 2). The most common cytological pattern observed was maturation arrest, identified in 49 patients (35.0%), indicating a halt in germ cell development at various stages. Normal spermatogenesis and hypospermatogenesis were each noted in 41 patients (29.3%), suggesting partial preservation of spermatogenic activity in a subset of cases. Testicular atrophy was seen in 7 patients (5.0%), characterized by marked reduction in germ cell population and parenchymal shrinkage. A small number of cases demonstrated complete absence of spermatogenesis (0.7%) and other rare findings (0.7%). **Table 3** details the cytological spectrum observed among azoospermic patients, including the proportion of cases showing normal spermatogenesis, hypospermatogenesis, maturation arrest, testicular atrophy, and absence of spermatogenesis. Fine-needle aspiration cytology (FNAC) of testicular aspirates revealed diverse cytological patterns among the 140 azoospermic patients (**Table 3**). Maturation arrest was the most prevalent finding, observed in 49 cases (35.0%), followed by normal spermatogenesis and hypospermatogenesis, each noted in 41 cases (29.3%). Testicular atrophy was identified in 7 patients (5.0%), while complete absence of spermatogenesis was documented in 1 patient (0.7%). Rare or miscellaneous findings accounted for another 0.7%. These results indicate that while a considerable proportion of azoospermic patients still retain partial spermatogenic activity, a significant number of exhibit varying degrees of germ cell maturation arrest or testicular degeneration, emphasizing the heterogeneity of testicular cytology in cases of male infertility. **Table 4** correlates the gross appearance of FNAC aspirates, such as scanty, whitish, findings, providing insight into how aspirate characteristics relate to testicular pathology**.** The appearance of FNAC aspirates varied among diagnostic categories (**Table 4**). Whitish or semifluid material was frequently associated with cases showing normal or reduced spermatogenesis, while scanty material was commonly noted in maturation arrest and hypospermatogenesis. Blood-stained aspirations were occasionally seen, particularly in cases with active spermatogenesis or germ cell arrest. These patterns indicate a relationship between the gross characteristics of aspirates and the underlying histo-cytological status of the tests. A trend toward statistical significance was noted ($p \approx 0.05$), suggesting that subnormal testosterone levels may be associated with a higher proportion of maturation arrest and hypospermatogenesis. **Table 5** demonstrates the relationship between serum testosterone levels and cytological findings in azoospermic patients. Among those with subnormal testosterone levels (n = 99), the most frequent cytological patterns were maturation arrest (39 cases; 39.4%) and hypospermatogenesis (32 cases; 32.3%), followed by normal spermatogenesis (21 cases; 21.2%). In contrast, patients with normal testosterone levels (n = 41) showed a higher proportion of normal spermatogenesis (20 cases: 48.8%), with fewer cases of maturation arrest and hypospermatogenesis. The Chi-square test revealed a borderline statistically significant association between testosterone level and cytological pattern ($\chi^2 = 10.83$, df = 5, p = 0.056). This finding suggests that lower testosterone levels were more frequently associated with impaired spermatogenic ativity, particularly maturation arrest and hypospermatogenesis, whereas normal testosterone levels correlated more often with preserved spermatogenesis. Logistic regression revealed that testosterone level was a significant independent predictor of abnormal spermatogenesis (p = 0.008) (**Table 6**). Each unit decrease in testosterone increased the odds of abnormal cytology by approximately twofold (OR = 0.50, 95% CI: 0.31–0.83). Age and LH/FSH levels were not significant predictors in the model. **Figures 2–7** depict cytological features of various spermatogenic patterns at low-power magnification (100×). **Figures 2 (A, B)** show normal spermatogenesis with orderly maturation of germ cells from spermatogonia to spermatozoa. **Figures 3 (A, B)** demonstrate hypospermatogenesis, characterized by a reduced number of germ cells with maintained maturation sequence. **Figure 4** illustrates early maturation arrest, with interruption at the level of primary spermatocytes, while **Figures 5 (A, B)** show late maturation arrest at the spermatid stage with absence of mature spermatozoa. **Figure 6** represents Sertoli cell-only syndrome, with seminiferous tubules lined exclusively by Sertoli cells and absence of germ cells. **Figure 7** shows testicular atrophy with complete maturation arrest, characterized by reduced tubular size, thickened basement membrane, and lack of active spermatogenesis.

Table 1: Semen Analysis and hormonal profile of the study participants (n=140)

Parameter	Category	No. of Patients	Percentage (%)
Semen analysis	Azoospermia	140	100%
LH/FSH Level	Normal	140	100%
Testosterone Level	Normal	41	29.3%
Serum Testosterone	Subnormal	99	70.7%

Table 2: Urogenital Findings Based on Cytological Interpretation.

Urogenital Finding	Number of Patients	Percentage (%)
Hypospermatogenesis	41	29.3
Normal spermatogenesis	41	29.3
Maturation arrest	49	35.0
Testicular atrophy	7	5.0
Sertoli cell only	1	0.7
Others	1	0.7
Total	**140**	**100**

Table 3: Distribution of Cytological Patterns (FNAC Findings)

Cytological Pattern	Number of Patients	Percentage (%)
Normal spermatogenesis	41	29.3
Hypospermatogenesis	41	29.3
Maturation arrest	49	35.0
Testicular atrophy	7	5.0
Sertoli cell only	1	0.7
Others	1	0.7
Total	**140**	**100**

Table 4: Relationship Between FNAC Aspirate Appearance and Cytological Diagnosisabscess

Aspirate Appearance	Normal Spermatogenesis	Hypospermatogenesis	Maturation Arrest	Testicular Atrophy	Sertoli cell only	Others
Scanty material	Present (most cases)	Present	Present	Occasional	Rare	–
Whitish / Semifluid material	Common	Common	Frequent	Few	–	–
Blood-stained aspirate	Occasional	Occasional	Occasional	–	–	–
Not recorded / unclear	Present in small number	Present in small number	Present	Few	–	–

Table 5: Association between Testosterone Level and Cytological Pattern

Cytological Pattern	Normal Testosterone (n = 41)	Subnormal Testosterone (n = 99)	Total	Chi Square	P value
Normal Spermatogenesis	20	21	41		
Hypospermatogenesis	9	32	41		
Maturation Arrest	10	39	49	10.53	0.056
Testicular Atrophy	1	6	7		
Sertoli cell only	1	0	1		
Others	0	1	1		
Total (n)	**41**	**99**	**140**		

Table 6: Binary Logistic Regression Analysis for Predictors of Abnormal Spermatogenesis

Predictor Variable	β (Coefficient)	SE	Wald χ^2	p-value	Odds Ratio (OR)	95% CI for OR
Age (years)	0.042	0.028	2.23	0.135	1.04	0.99–1.10
Testosterone (ng/mL)	–0.685	0.260	6.93	**0.008**	**0.50**	0.31–0.83
LH/FSH (IU/L)	0.058	0.074	0.62	0.431	1.06	0.92–1.21
Constant	0.982	0.911	—	0.289	—	—

Nagelkerke $R^2 = 0.24$; Model $\chi^2 = 15.67$, df = 3, p = 0.001; Classification accuracy = 78.6%

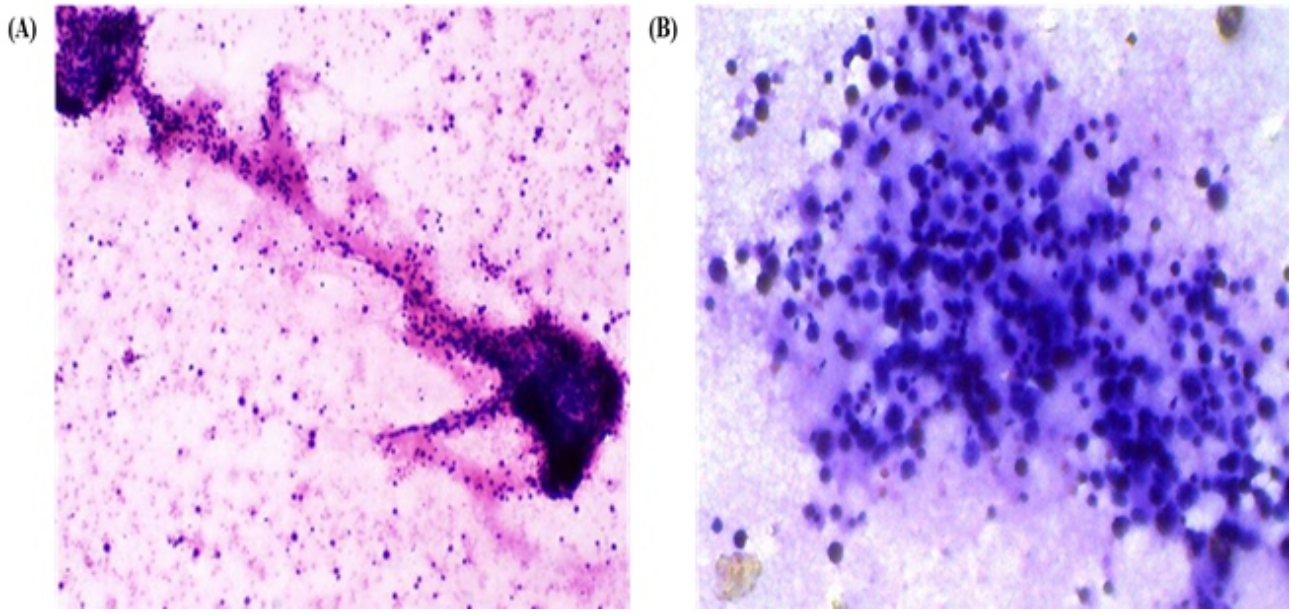

Figure 2: (A and B) Low-power view (100 X) of normal spermatogenesis.

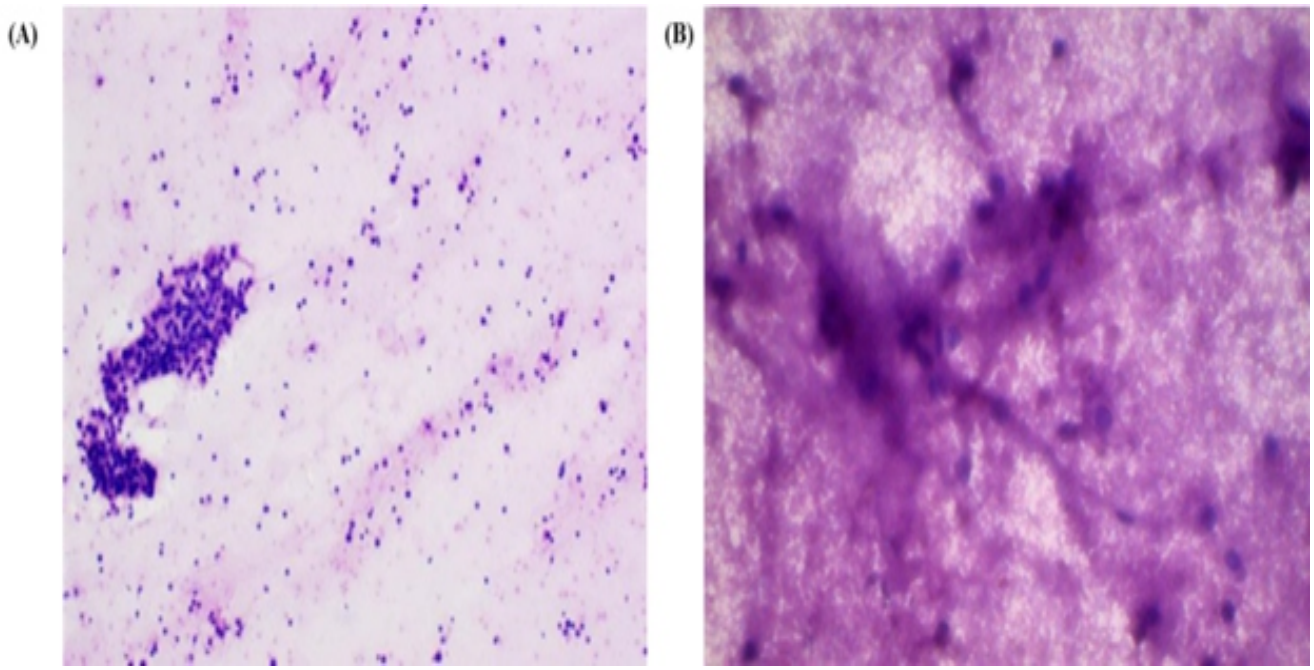

Figure 3: (A & B) Low-power view (100X) of hypo-spermatogenesis.

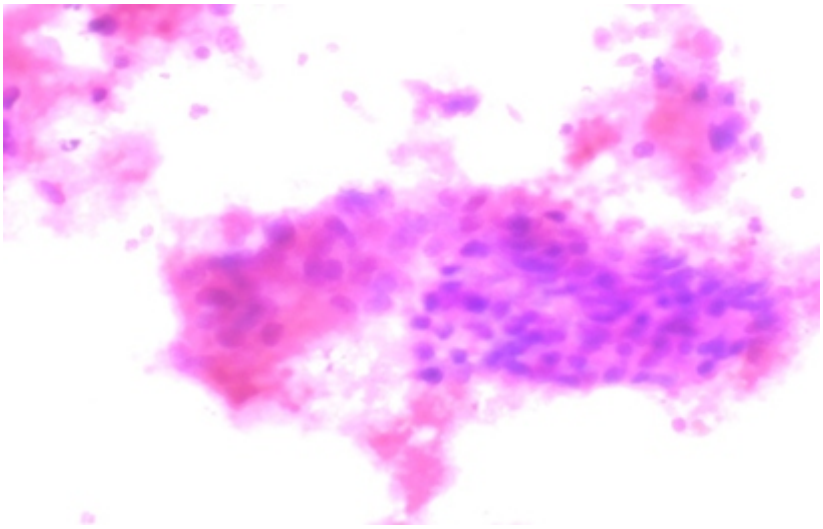

Figure 4: Low-power view (100X) of early maturation arrest.

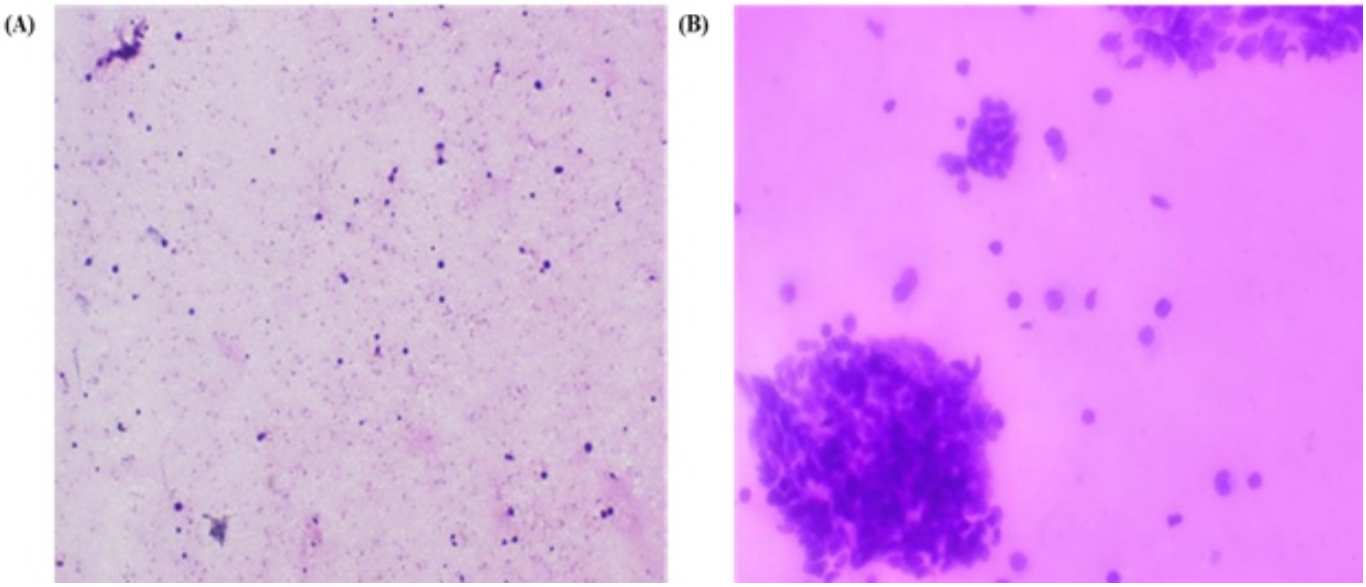

Figure 5: (A & B) Low-power view (100X) of late maturation arrest.

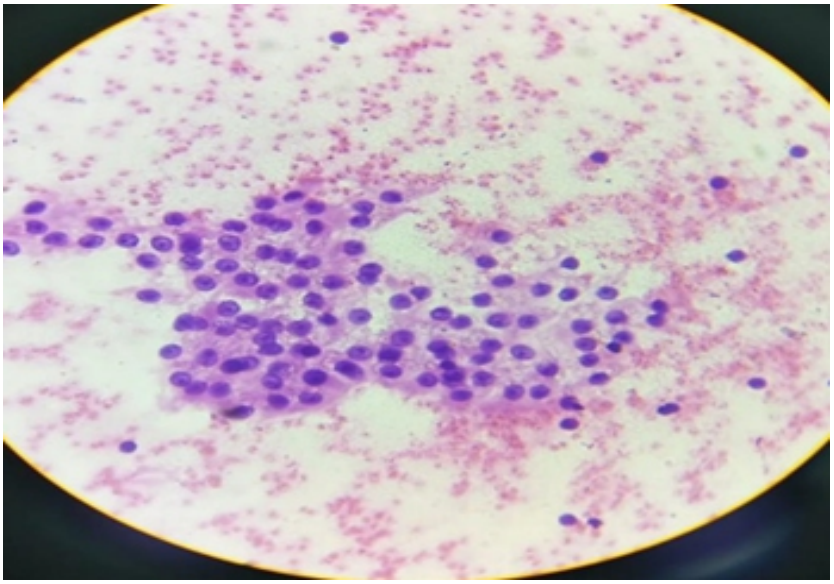

Figure 6: Sertoli cell only syndrome.

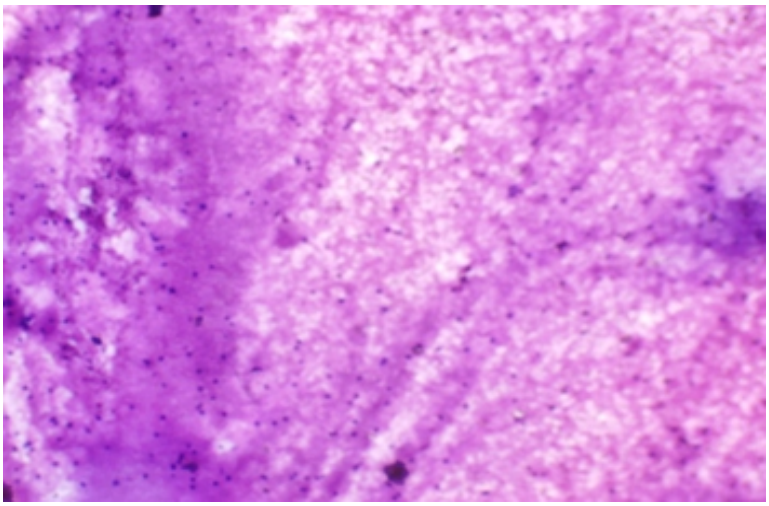

Figure 7: Testicular atrophy with complete maturation arrest.

DISCUSSION

Testicular fine-needle aspiration cytology (FNAC) has consistently demonstrated its value as a minimally invasive, cost-effective, and reliable first-line investigation in the evaluation of male infertility. Early evidence from large series established that FNAC provides a clear morphological distinction between normal spermatogenesis, hypospermatogenesis, maturation arrest, Sertoli-cell-only syndrome (SCOS), and testicular atrophy [1,2].

The 2018 cytology histology correlation study further strengthened this evidence, demonstrating that FNAC could detect the entire spectrum of spermatogenic activity with good accuracy, achieving a 67.4% correlation rate despite a significant number of "insufficient" smears, attributed largely to atrophic or fibrotic testes (3). The Indian Journal of Pathology & Oncology study (2017) also reported strong FNAC-biopsy agreement (≈ 86%), and highlighted that maturation arrest and SCOS were amongst the most common cytologic patterns encountered. This study additionally introduced quantitative cytological indices- Spermatic Index (SI), Sertoli Cell Index (SEI), and Sperm-Sertoli Cell Index (SPSEI)- which provided objective numerical parameters to differentiate borderline patterns such as hypospermatogenesis versus late maturation arrest [4]. These quantitative indices were validated & expanded in the most recent 2025 study, which demonstrated an exceptionally high cytology histology concordance of 97.9%, establishing that FNAC, when supported with standardized cell counts and indices, approaches diagnostic accuracy comparable to open biopsy [5]. The study showed that SI and SPSEI sharply declined from normal spermatogenesis to hypospermatogenesis and became zero in maturation arrest or SCOS, whereas SEI increased progressively from normal through hypospermato-genesis to SCOS. Such objective gradients reinforce FNAC as a powerful stratification tool that can accurately predict underlying histology and guide clinical decision-making regarding assisted reproductive technologies such as TESE or ICSI [5].

FNAC also offers significant procedural advantages: it permits sampling from multiple testicular sites, thereby reducing sampling errors inherent to biopsy, especially in cases of focal or patchy spermatogenesis [1,2]. It is quick, well-tolerated, repeatable, and avoids scary or postoperative complications, especially valuable when repeated sampling may be required before sperm retrieval procedures. However, limitations persist. FNAC does not assess tubular architecture, basement membrane integrity, interstitial fibrosis, Leydig cell hyperplasia, or vascular/inflammatory pathology, elements that may be critical in certain etiologies of infertility [3,4]. Additionally, non-diagnostic or inadequate smears may occur, particularly in an atrophic testis, potentially leading to underestimation of spermatogenic activity. Occasional discordance also arises when cytology suggests hypospermatogenesis, but biopsy reveals maturation arrest, or vice versa (4). Therefore, while FNAC is an excellent first line modality, open biopsy retains a complementary role when architectural detail is essential, cytology is inconclusive, or clinical findings remain discordant with cytological impressions.

Overall, integrating evidence across two decades of literature, including the most recent high-quality 2025 data, supports that testicular FNAC, especially when enhanced with quantitative cytological indices, is a robust, minimally invasive, and clinically meaningful tool for evaluating spermatogenesis in male infertility. It is best positioned as the initial diagnostic approach, with open biopsy reserved for non-diagnostic aspirations, unclear cases, or when detailed histo-architecture is required.

CONCLUSION

Testicular FNAC is a reliable, minimally invasive, and cost-effective first line tool for evaluating spermatogenesis in male infertility. When combined with quantitative cytological indices, it accurately identifies patterns such as normal spermatogensis, hypo-spermatogenesis, maturation arrest, and Sertoli-cell only syndrome, with diagnostic concordance approaching that of open

biopsy. FNAC's advantages-multi-site sampling, repeatability, and minimal morbidity-make it particularly valuable for guiding clinical decisions, including assisted reproductive procedures. Nonetheless, open biopsy remains important in cases requiring architectural assessment or when FNAC yields non-diagnostic results. Overall, FNAC should be considered the initial diagnostic modality, reserving biopsy for selected, complex cases.

CLINICAL SIGNIFICANCE

The clinical significance of this study lies in its potential to bridge the gap between research findings and practical healthcare applications. It emphasizes the importance of trans-lating scientific observations into meaningful improve-ments in patient care, diagnosis, and treatment outcomes. By highl-ighting real-world relevance, the study contributes to evidence-based medical practice and supports informed clinical decision-making. Ultimately, the findings aim to enhance patient quality of life, optimize therapeutic strategies, and promote better disease management in clinical settings.

ABBREVIATIONS

FNAC: Fine Needle Aspiration Cytology
OA: Obstructive Azoospermia
NOA: Non-Obstructive Azoospermia
MA: Maturation Arrest

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AUTHOR CONTRIBUTIONS

All authors significantly contributed to the study conception and design, data acquisition, or data analysis and interpretation. They participated in drafting the manuscript or critically revising it for important intellectual content, consented to its submission to the current journal, provided final approval for the version to be published, and accepted responsibility for all aspects of the work. Additionally, all authors meet the authorship criteria outlined by the International Committee of Medical Journal Editors (ICMJE) guidelines.

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CONFLICT OF INTEREST

Authors declared that there is no conflict of interest.

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All necessary consent & approval was obtained by authors.

CONSENT FOR PUBLICATION

All necessary consent for publication was obtained by authors.

DATA AVAILABILITY

All data generated and analyzed are included within this research article. The datasets utilized and/or analyzed in this study can be obtained from the corresponding author upon a re-asonable request.

USE OF ARTIFICIAL INTELLIGENCE (AI) & LARGE LANGUAGE MODEL (LLM)

The authors confirm that no AI & LLM tools were used in thewriting or editing of the manuscript, and no images were altered or manipulated using AI & LLM.

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This article serves as an important educational tool for the scientific community, offering insights that may inspire future research diretions. However, they should not be relied upon independently when making treatment decisions or developing public health policies.

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