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Pathology Section

Histopathological Patterns of Testicular Biopsy in Male Infertile Patients: A Cross-sectional Study

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ABSTRACT

Introduction: The management of male infertility has undergone rapid changes with advancements in assisted reproductive techniques such as testicular sperm aspiration and microdissection, testicular sperm extraction (MicroTESE). However, testicular biopsy remains an important tool for diagnosing and managing male infertility, as well as assessing spermatogenesis levels.

Aim: To examine the histological patterns of testicular biopsies in male patients with infertility.

Materials and Methods: This cross-sectional study was conducted at the Department of Pathology, ESIC Super Specialty Hospital, Hyderabad, Telangana, India from May 2012 to May 2023. A total of 141 Tru-Cut biopsies from patients with male infertility were included based on predefined inclusion and exclusion criteria. Histopathologists labeled and reported the slides, noting the pattern of histopathology and level of spermatogenesis according to Johnson's criteria. The data was analysed for percentages and mean values.

Results: The majority of patients 43 (30.5%) exhibited normal histopathology, followed by Sertoli cell-only syndrome in 37 (26.24%) patients, and complete spermatogenic arrest in 14 (9.93%) patients. The least common pattern observed was incomplete spermatogenic arrest. Testicular atrophy was observed in 13 (9.22%) patients.

Conclusion: This study highlights the importance of understanding the histological patterns observed in testicular biopsies, as it provides valuable insights to clinicians regarding the likelihood of obtaining spermatozoa for testicular sperm extraction in infertile males.

Keywords: Histopathology of testis, Johnson's criteria, Testicular atrophy

INTRODUCTION

Infertility is a prevalent global problem that affects 15% of couples trying to conceive, with approximately 30% of cases attributed to male partner abnormalities [1]. A comprehensive evaluation of male infertility involves a thorough history, physical examination, semen analysis, biochemical investigations, and testicular biopsy [2]. Biopsy is indicated in azoospermic or severely oligozoospermic patients, as it helps differentiate obstructive from non obstructive causes and assesses the level of spermatogenesis in the testis. The causes of azoospermia can be classified as pretesticular, testicular, and post-testicular [3,4]. Pretesticular azoospermia is primarily hormone-related at the level of the hypothalamus, pituitary, or adrenals, resulting from insufficient testicular stimulation due to low Follicle Stimulating Hormone (FSH) levels [5,6]. Testicular causes of azoospermia can be attributed to morphological and structural testicular abnormalities [7]. Congenital absence or atrophy of the testis, cryptorchidism, Klinefelter's syndrome, and Sertoli cell-only syndrome are some of the causes, along with acquired factors such as trauma, surgery, orchitis, radiation, and carcinoma [8-10]. Post-testicular azoospermia is mainly due to obstructive causes, affecting around 7-50% of males. It is commonly associated with conditions like congenital absence of the vas deferens, cystic fibrosis, vasectomy, or ejaculation disorders such as retrograde ejaculations. The cause is unknown in idiopathic azoospermia [6].

In addition to these causes, infertility can result from arrested or reduced spermatogenesis, which may have genetic, hormonal, thermal, or toxic drug-related origins [8]. Hypospermatogenesis can be caused by factors such as toxic drugs, diabetes mellitus, varicocele, hypothyroidism, radiation exposure, and excessive heat [11].

Testicular biopsy has gained significance in the evaluation of male infertility, particularly with the advancement of technologies like MicroTESE, as it aids in further management, counselling, and providing valuable information to the patient [12]. Compared to MicroTESE, testicular biopsy provides a larger tissue sample for histopathological examination, offering a more accurate assessment of the level of spermatogenesis and the presence of spermatids or sperm. These findings can be correlated with clinical features, hormone levels, and karyotyping, facilitating appropriate fertility treatment for patients [13]. This study aims to differentiate cases of male infertility based on various histopathological patterns.

MATERIALS AND METHODS

This cross-sectional study examined cases of testicular biopsies from male patients with infertility, retrieved from the archives of the Department of Pathology, ESIC Superspeciality and Medical College Hospital, Hyderabad, Telangana, India. The data collection period spanned from May 2012 to May 2023, and the selected age group for the study was 20 to 50 years. The study was approved by Institutional Ethical Committee (IEC) and was assigned the IEC number ESICMC/SNR/IEC-F511/03-2023.

Inclusion criteria: All tru-cut testicular biopsies received in the Department of Pathology with infertility as the clinical history were included in the study.

Exclusion criteria: Inadequate tissue (less than 20 seminiferous tubules cross-section), testicular biopsy with neoplasia, and autolysed tissue were excluded from the study.

The biopsy procedure involved removing a small piece of tissue from patients under anaesthesia. The tissues were then sent to the lab in Bouin's fluid, which consists of 1.5% picric acid, 9% formaldehyde, and 5% acetic acid. Formalin was not used as a fixative due to its disruptive effect on the testis tissue architecture. The tissue samples were labelled and fixed for 12 hours, processed, and embedded in paraffin. Sections were cut at 4 microns using a microtome, followed

by staining with haematoxylin and eosin. The pathologist reported the findings based on the Johnsen scoring criteria [4] and described the histological pattern.

Sections containing 20 or more seminiferous tubules were considered adequate for histopathological examination [13].

The following parameters were studied:

1. Number of tubules.
2. Tubular basement membrane thickness.
3. Presence of hyalinised tubules.
4. Prominence of Sertoli cells.
5. Maturation pattern of germ cells.
6. Cells in the interstitium.

A quantitative histological grading system called the Johnsen score [4] was used to assess the level of sperm maturation, graded between 1 and 10. In a normal adult male, atleast 60% of the tubules should have a score of 10. This scoring system evaluates the degree of spermatogenesis.

The scoring criteria are as follows:

1. No germ cells or Sertoli cells present.
2. No germ cells present.
3. Only spermatogonia present.
4. Only a few spermatocytes present.
5. No spermatozoa or spermatids present, but many spermatocytes present.
6. Only a few spermatids present.
7. No spermatozoa but many spermatids present.
8. Only a few spermatozoa present.
9. Many spermatozoa present but disorganised spermatogenesis.
10. Complete spermatogenesis and perfect tubules.

In this study, testicular histology was categorised according to Rosai and Ackerman's classification [4]:

1. Normal spermatogenesis.
2. Hypospermatogenesis.
3. Sertoli cell-only syndrome.
4. Complete maturation arrest.
5. Incomplete maturation arrest.
6. Atrophic testis.
7. Mixed pattern when two or more patterns were observed.

The total number of testicular biopsies submitted to the Department of Pathology at ESIC Superspeciality Hospital, Sanathnagar was 141. The biopsies were processed and stained with H&E, and the morphology was analysed in terms of tubules, basement membrane, and interstitium.

STATISTICAL ANALYSIS

The collected data was analysed for frequency with percentages and mean.

RESULTS

The age groups of the studied cases ranged from 21 to 46 years, with a mean age of 33.5 years. The majority of patients belonged to the 21-30 year age group, followed by the 31-40 year age group [Table/Fig-1].

The Johnsen score for the biopsies is shown in [Table/Fig-2]. A mixed pattern was observed in 23 cases (16.31%). The biopsies were classified into seven types [Table/Fig-3]. Normal spermatogenesis was seen in 43 (30.50%) cases, followed by Sertoli cell-only syndrome in 37 (26.24%) cases.

Age (years)	n (%)
3rdDecade (21-30)	69 (48.94)
4thDecade (31-40)	67 (47.52)
5thDecade (41-50)	05 (3.54)

[Table/Fig-1]: Age wise distribution of cases (N=141).

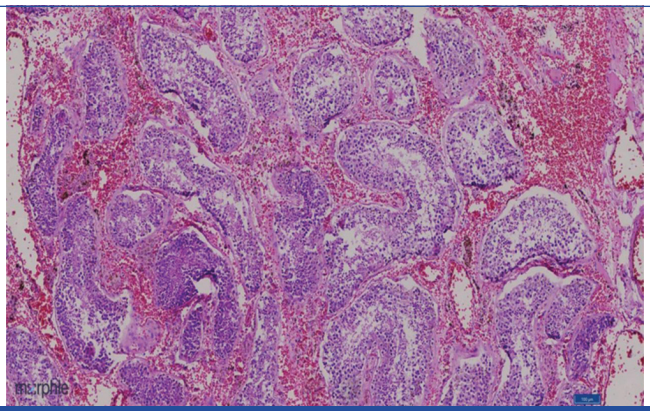
Johnsen's criteria	n (%)
10	36 (25.53)
9	7 (4.96)
8	7 (4.96)
7	9 (6.38)
6	3 (2.12)
5	2 (1.41)
4	2 (1.41)
3	2 (1.41)
2	37 (26.24)
1	13 (9.21)
Mixed pattern	23 (16.31)

[Table/Fig-2]: Distribution of cases based on Johnsen's criteria.

Histopathology	n (%)
Normal	43 (30.49)
Mixed pattern	23 (16.31)
Sertoli cell only syndrome	37 (26.24)
Complete spermatogenic arrest	14 (9.92)
Incomplete spermatogenic arrest	04 (2.83)
Atrophic testis	13 (9.21)
Hypospermatogenesis	07 (4.96)

[Table/Fig-3]: Distribution of histopathological patterns.

Azoospermia on semen analysis and normal spermatogenesis on histology indicate obstruction in some part of the duct system [Table/Fig-4]. All germ cell stages, including mature spermatozoa, are present in normal spermatogenesis.



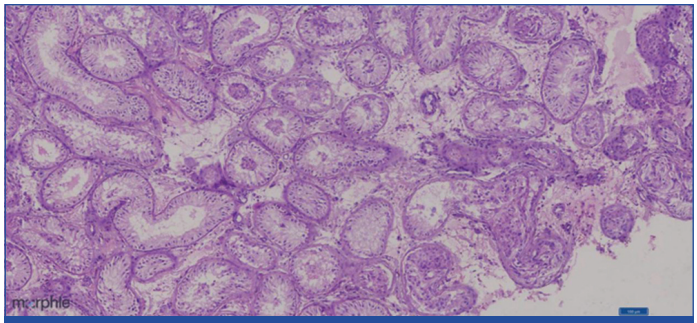
[Table/Fig-4]: Showing normal spermatogenesis (H&E,40x).

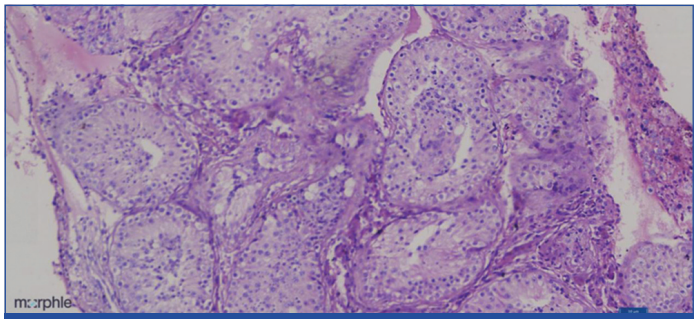
In hypospermatogenesis (4.96%), all germ cell stages, including spermatozoa, are present, but there is a distinct decrease in the number of germ cells.

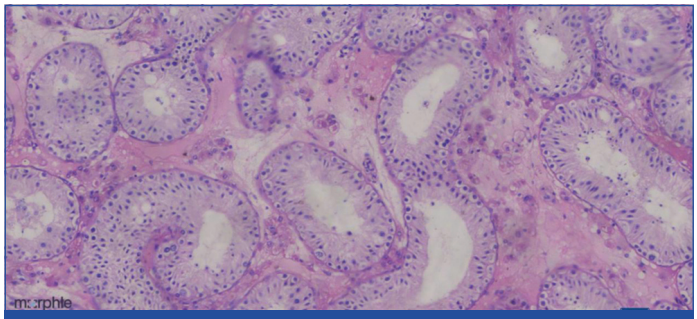
Sertoli cell-only syndrome, as the name implies, showed only Sertoli cells without spermatogenesis. The Sertoli cells were oval in shape and arranged perpendicular to the basement membrane [Table/Fig-5].

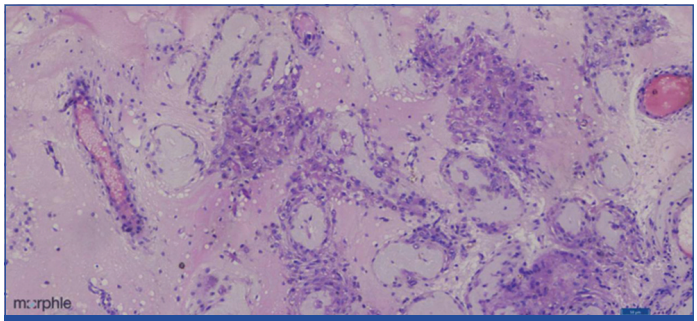
In both types of maturation arrest, germ cells are seen only up to the spermatocytic stage, resulting in mostly azoospermia in semen. The most common stage at which maturation arrest was observed was the primary spermatocyte stage. This is also referred to as complete spermatogenic arrest (9.93%) [Table/Fig-6,7].

In cases of testicular atrophy (9.22%), there is an absence of seminiferous tubules with tubular sclerosis [Table/Fig-8].

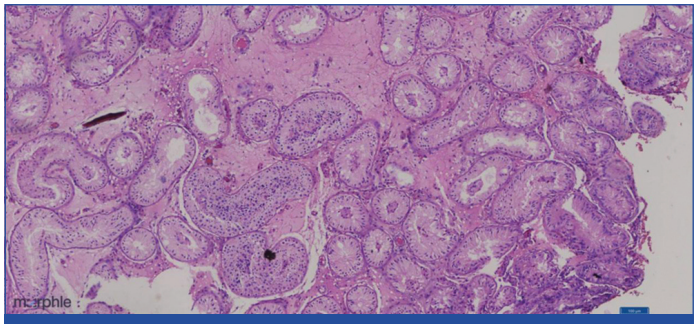

[Table/Fig-5]: Showing sertoli cell-only syndrome (H&E,40x).

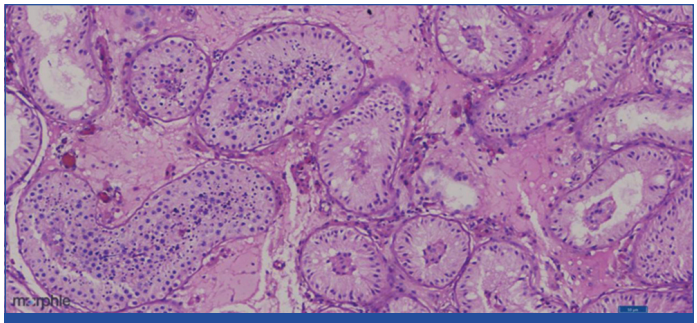

[Table/Fig-6]: Showing incomplete spermatocytic arrest (H&E,40x).


[Table/Fig-7]: Showing complete spermatocytic arrest (H&E,40x).


[Table/Fig-8]: Showing testicular atrophy (H&E,40x).

In the mixed pattern, different stages of spermatogenesis or tubular sclerosis often coexist in the same biopsy, showing varying patterns. Out of the 23 cases with a mixed pattern, 11 cases showed Sertoli cell-only syndrome with hypospermatogenesis, three cases showed Sertoli cell-only syndrome with normal spermatogenesis [Table/Fig-9,10], seven cases showed atrophic testis with hypospermatogenesis, and two cases showed atrophy with normal spermatogenesis.


[Table/Fig-9]: Showing a low-power view of the mixed pattern (H&E,10x).


[Table/Fig-10]: Showing a high-power view of the mixed pattern (H&E,40x).

In all the biopsies, the interstitium was mostly normal. Patterns of Leydig cells in the interstitium were noted, such as normal Leydig cells, Leydig cell clusters (<10 cells), and Leydig cell hyperplasia (if more than 10-20 cells per cluster). In the present study, 3 (2.12%) cases showed Leydig cell clusters, and one showed Leydig cell hyperplasia.

DISCUSSION

Male infertility is often associated with issues in sperm production and function. According to the World Health Organisation (WHO), over 70 million couples worldwide experience infertility symptoms [14]. However, advancements in technology have expanded options for infertile couples. Diagnostic testicular biopsies can reliably predict the level of spermatogenesis, leading to successful sperm extraction techniques like micro TESE. In this study, the average age of the patients was 33.5 years, which differs from previous studies reporting lower mean ages [11,15-20]. These variations could be attributed to demographic and cultural factors, but no definite conclusions can be drawn.

The most common histopathological pattern observed in this study was normal spermatogenesis (30.50%), indicating an obstructive aetiology. This suggests that viable sperm are present in the testicular tissue, but a blockage prevents their presence in the semen. Obstruction can be caused by various factors such as varicocele, testicular torsion, or infections. Assisted reproductive techniques offer favourable outcomes for these patients. Similar findings have been reported in previous studies [15,21].

The second most common pattern observed was Sertoli Cell-Only syndrome (SCO) in 26.24% of cases. SCO is irreversible and can be caused by underlying conditions like cryptorchid testes, chemo or radiotherapy, orchitis, or structural abnormalities in the long arm of the Y chromosome [15,20,22,21].

Other histological features observed include spermatic arrest, which was the most common in some studies [13,22-24], seminiferous tubule hyalinisation [25], hypospermatogenesis [17,11], and a mixed pattern [20]. The presence of these patterns requires careful examination and reporting in testicular biopsies. Sperm retrieval yield varies depending on the histological pattern.

The variations in histological patterns among studies may stem from genetic, environmental, demographic, and cultural differences. Further investigation is needed to validate these results and understand the underlying factors for these variations.

Limitation(s)

The serum hormone levels, including Luteinizing Hormone (LH), FSH, testosterone, and prolactin, were not evaluated. Additionally, chromosomal analysis of the patients was not conducted.

CONCLUSION(S)

Testicular biopsy is an important investigation in the evaluation of male infertility and aids in the clinical management of these patients. The histopathology patterns identified in the biopsy can indicate whether spermatogenesis is preserved or disrupted, providing valuable information for sperm extraction techniques such as in-vitro

fertilisation and intracytoplasmic sperm injection. Moreover, a biopsy can differentiate between obstructive and non obstructive azoospermia, assisting clinicians in planning reconstructive surgery for patients with obstructive azoospermia and normal spermatogenesis on biopsy. Thus, the histological evaluation of testicular biopsy plays a crucial role in guiding infertile males towards successful parenthood.

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