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Histopathological Variation in Testicular Biopsy Done for Primary Infertility Showing Azoospermia

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Abstract

Male infertility contributes to 20% of all infertility cases. It is estimated that 1% of the total male population and 10% of men seeking infertility treatment are affected by testicular failure. In patients with azoospermia, testicular biopsy is important to study the histopathological patterns of the testes. Testicular biopsy specimen from patients with azoospermia from Sudha Medical College and Private diagnostic center from July 2022 to July 2025 were included in the present study. All were unilateral right testicular biopsies. The number of 26 cases of testicular biopsy of the right side was done to find out the etiology of infertility. All biopsies were collected in Boin's fluid and studied for histopathological patterns and categorization by Johnson grading. The most common pattern was mixed pattern, which was followed by Sertoli cell-only syndrome. The study was done to know the variations in the histological patterns and subsequently to decide on treatment.

Keywords: Azoospermia, Histological patterns, Primary infertility, Testicular biopsy, Variations

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Introduction

Male infertility contributes to 20% of all infertility cases.[1] Infertility is defined as the inability to conceive after 1 year of unprotected intercourse.[2] It is estimated that 1% of the total male population and 10% of men seeking infertility treatment are affected by testicular failure[3] In patients with azoospermia, testicular biopsy is important to study the histopathological patterns of the testes. Azoospermia is characterized by the absence of sperm in the ejaculate and can be classified as obstructive or non-obstructive.[4] Men with obstructive causes have preserved spermatogenesis, and they can benefit from sperm retrieved by epididymal or testicular extraction.[5] In men with non-obstructive cause, accurate etiological diagnosis is needed to increase the chances of subsequent sperm recovery for intracytoplasmic sperm injection (ICSI). A precise etiological diagnosis is based on histopathological findings, to aid in isolation and *in vitro* culture of germ cells from spermatogonia to early round spermatids.[6] As spermatogenesis takes place in testes, testicular biopsy is done and categorized into various histopathological patterns and presence of sperms in testes[7] which provides valuable information regarding prognosis and treatment.[8] To evaluate male infertility, it is important to have a proper history and physical examination along with semen analysis and hormonal assay. In cases of azoospermia, apart from testicular biopsy, other tests include anti-sperm antibodies, transrectal ultrasonography, vasography, genetic studies, and hormonal profile.

The present study aimed to evaluate the histopathological patterns of testicular biopsy from infertile males and categorize each according to the Modified Johnson scoring system.

Material and Methods

Testicular biopsy specimens from Sudha Medical College and a private diagnostic center from July 2022 to July 2025 were included in the study. All were unilateral right testicular biopsies. Inclusion criteria for testicular biopsy were infertility, which is defined as failure to achieve a successful pregnancy after >12 months of regular unprotected intercourse, and azoospermia in at least two seminal analyses, defined as the absence of spermatozoa in ejaculated semen samples after specimen centrifugation and pellet examination.

The biopsies were performed under spermatic cord block anesthesia, and tissue samples measuring approximately 10 × 6 mm in size were cut longitudinally.

Testicular biopsy was preserved in Boiun's fluid, and sections were stained with H&E stain and examined under the microscope. Biopsy can be processed using glutaraldehyde for fixation and glycol methacrylate (GMA)[9] or epoxy resin for embedding, which improves tissue preservation and microscopic resolution. GMA embedding has advantages such as it is fast to process, hydrosoluble, easy to handle, infiltration and polymerization at room temperature, thin sections with less distortion and artifacts, and better resolution under light microscopy.[10]

Adequate testicular biopsy for infertility should be 3 mm in any one dimension, containing 3–5 lobules or 9–20 seminiferous tubules with intervening septa.

All sections were evaluated initially with 200 × magnification for a general description of the testicular parenchyma and then in 400 × magnification for a more detailed study of the seminiferous epithelium cells. Various histopathological patterns were evaluated and categorized into different histopathological patterns[11] and then evaluated for as per the Modified Johnson's scoring from 1 to 10.[12]

There are various histopathological patterns of testicular biopsy.

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1. Normal spermatogenesis: The seminiferous tubules are lined by a thin basement membrane, and the germinal epithelium shows normal progression from spermatogonia to spermatocytes along with spermatids and spermatozoa.
2. Hypospermatogenesis: The germinal epithelium shows all the stages of germ cells, but the number is reduced.
3. Germ cell maturation arrest: At a specific cell stage, the process of spermatogenesis is arrested, usually at the level of primary or secondary spermatocytes.
4. Sertoli cell-only syndrome: The tubules contain only Sertoli cells and no other cells of spermatogenesis.
5. Seminiferous tubule hyalinization: The tubules have much-thickened basement membrane with a smaller diameter, along with tubular collagenization. There is no germinal epithelium.
6. Mixed pattern: There is variation in the histopathological pattern in the same testicular biopsy.
7. Discordant pattern: There is variation in the histopathological pattern of the right and left testes.

Biopsies were then evaluated for the Modified Johnson scoring from 1 to 10 as follows:

- Score 10: There is full spermatogenesis.
- Score 9: Incomplete spermatogenesis with many late spermatids.
- Score 8: There are <5 spermatozoa per tubule and a few late spermatids.
- Score 7: There are many early spermatids but no spermatozoa or late spermatids.
- Score 6: There are a few early spermatids but no spermatozoa or late spermatids.
- Score 5: There are many spermatocytes but no spermatozoa or spermatids.
- Score 4: There are a few spermatocytes but no spermatozoa or spermatids.
- Score 3: There are only spermatogonia.
- Score 2: Only presence of Sertoli cells and no germinal epithelial cells.
- Score 1: There is no seminiferous epithelium.

In each case, findings were recorded.

Results

A total of 26 cases of testicular biopsies were evaluated for histological categorization. All patients had azoospermia with an age range from 24 years to 51 years.

The most common histological pattern was mixed, 26.92% of cases, followed by Sertoli cell-only syndrome, 23.07% of cases [Figures 1-6].

Table 1 shows the histological classification of testicular biopsies in infertile males ($n = 26$).

Table 2 shows the mixed histopathological patterns in the same testicular.

All cases were further classified based on the Modified Johnson scoring system, as shown in Table 3.

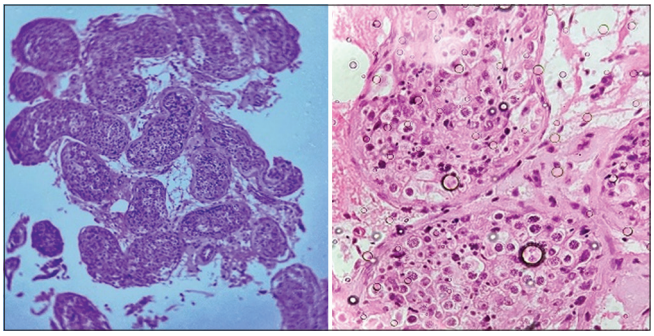

Figure 1: Normal spermatogenesis

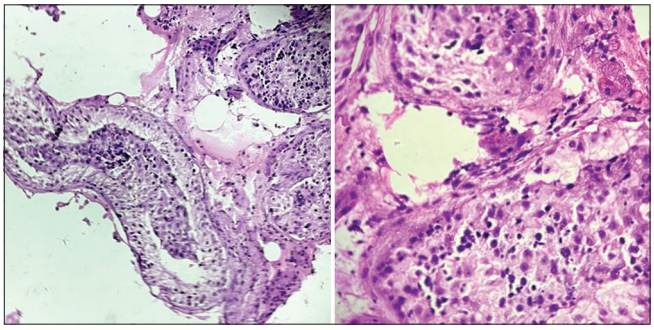

Figure 2: Germ cell maturation arrest

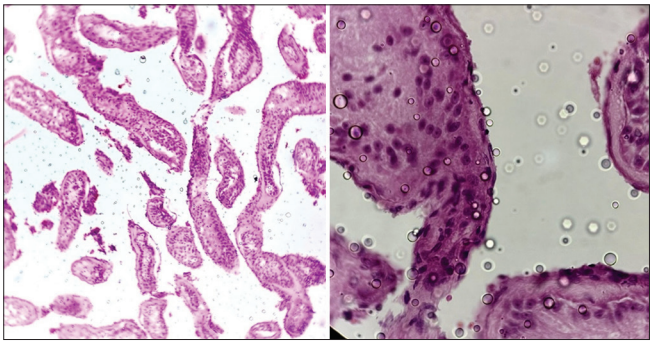

Figure 3: Sertoli cell-only syndrome

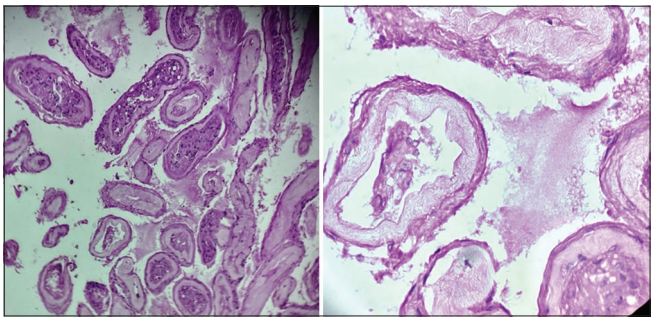

Figure 4: Seminiferous tubule hyalinization

Discussion

Male infertility accounts for 20% of all cases of infertility. The causes can be divided into three categories – pretesticular, testicular, and post-testicular.

Pre-testicular causes are extragonadal endocrine disorders originating in the hypothalamus, pituitary, and adrenals, which affect spermatogenesis through aberrant hormonal stimulation or suppression. Hypogonadotrophic hypogonadism can either be due to congenital Kallmann's syndrome or acquired due to trauma or tumors.[13] Various drugs that decrease FSH level and sperm motility.

Testicular causes are primary defects of the testes, congenital or secondary to environmental causes or other diseases, or

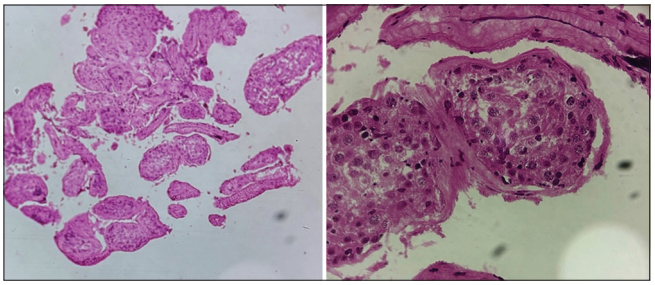

Figure 5: Mixed pattern

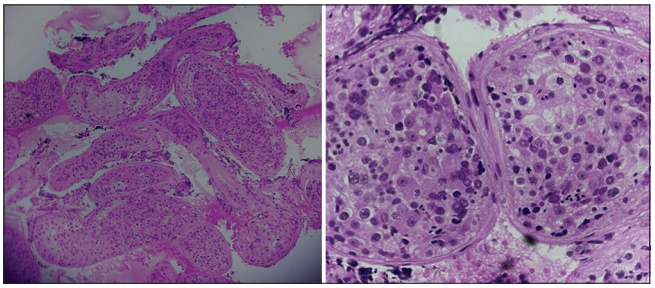

Figure 6: Basement membrane hyalinization

Table 1: Histological classification of testicular biopsies in infertile males

Sr. No.	*Histopathological classification*	*No. of cases*	*%*
1.	Normal spermatogenesis	03	11.53
2	Hypospermatogenesis	01	03.84
3	Germ cell maturation arrest	04	15.38
4	Sertoli cell-only syndrome	06	23.07
5	Seminiferous tubule hyalinization	05	19.23
6	Mixed pattern	07	26.92
7	Discordant pattern	00	00

Table 2: Mixed histopathological patterns in the same testicular biopsy (*n*=07)

Sr. No.	*Mixed histopathological patterns*	*No. of cases*
1	Sertoli cell-only syndrome and hyalinization arrest	03
2	Incomplete spermatogenesis with tubular hyalinization	01
3	Normal maturation with tubular hyalinization	01
4	Presence of mature and immature spermatozoa	02

Table 3: Modified Johnson scoring of testicular biopsy (*n*=26)

Modified Johnson scoring	*No. of case*	*%*
10	06	23.07
9	05	19.23
8	01	03.84
7	02	07.69
6	02	07.69
5	01	03.84
4	00	00.00
3	02	07.69
2	05	19.23
1	02	07.69

maldescended testes. Congenital causes are Klinefelter's syndrome, Y deletion. Acquired causes are radiotherapy, chemotherapy, torsion, and mumps orchitis.

Post-testicular causes are obstruction of the ducts leading away from the testes, which can be surgically reconstructed. Problem with ejaculation, lack of vas deferens, infections, hypospadias, ejaculatory duct obstruction, or impotence.[14]

This categorization of infertility etiology helps in taking corrective measures and withholding therapy in cases where a biopsy indicates poor prognosis for fertility.[15]

In the present study, 3 cases (11.53%) showed normal spermatogenesis, indicating duct obstruction as the cause of infertility. Among these, 23.07% were of Johnson score 10, and 19.23% were of score 9. Rashed *et al*. had 10% cases were of score 10 and 14% cases were of score 9.[16] Jamal and Mansoor reported 15% normal spermatogenesis, similar to the present study.[17] Hypospermatogenesis represented 3.84% of all cases, which is lower than the result in a study by Thomas 19%[18] and Al Rayees *et al*. 13% of cases.[19] In hypospermatogenesis, there is a good chance of isolating viable and intact spermatozoa which are capable of fertilization.

Germ cell maturation arrest represented 15.38% of all cases, which is similar to a study by Brannen and Roth who reported 12.5%,[20] Golgen and Al Rayees *et al*.[19] reported 11%. It is due to genetic or some toxic substances (antibiotics, chemotherapy, or radiotherapy) some liver or kidney disease. Sertoli cell syndrome in the present study has 23.07% cases. Rashed *et al*. 34%[16] and Al Rayees *et al*. reported 23.5% cases, which is similar to the present study.[19] It may be due to irradiation, cytotoxic drugs, cryptorchidism, or consanguity.

Wong *et al*.[21] reported 6% cases and Haddad *et al*.[22] reported 2.9%. In both these studies cases were low.

Tubular hyalinization 19.23% in the present study. All other studies reported a lower rate than Rashed *et al*.[16] reported 6%, Meinhard *et al*.[23] reported 6%, Al Rayees *et al*.[19] reported 6% cases of tubular hyalinization.

Mixed pattern was 26.92% in the present study. Abdullh and Bondagii reported 9 cases of mixed pattern.[7]

It is important to differentiate between obstructive and non-obstructive azoospermia because obstructive azoospermia has a cost-effective treatment option, such as microsurgical reconstruction of the obstructed ducts.

Testicular biopsy remains the standard test to characterize the severity of parenchymal damage and detect residual spermatogenesis in men with nonobstructive azoospermia and to predict sperm recovery for ICSI.[21] Biopsy tissue can be fixed in Bouin's fluid or G/GMA.

Bouin is a coagulated fixative, consisting of a mixture of different chemical compound, including picric acid which causes protein precipitation, resulting in tissue retraction and is explosive.[24]

Glutaraldehyde is non-coagulant fixative, forms molecular bridges between its aldehyde group and the amino groups of the protein, making them insoluble in the form of gel, reacts less intensely with lipid, carbohydrates and nucleic acids, so fixes the cell as a whole.[25]

In routine practice, Bouin's fluid is used as a fixative and slides are stained with H & E stain for histopathological examination.

Conclusion

The present study was conducted from July 2022 to July 2025. 26 cases of testicular biopsy of the right side were done to find out the etiology of infertility, by categorization of histopathology and Johnson grading. The most common pattern was the Mixed pattern, 2nd most common was Sertoli cell-only syndrome. There is

variation in the histological patterns in the same testicular biopsy. It is an important investigation to know the cause of infertility and hence the respective treatment.

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