Original Article

# Histopathological patterns of testicular biopsy in male infertility: A retrospective study from a tertiary care center in the western part of Saudi Arabia

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## Abstract

**Objective:** To identify and categorize various pathological changes seen in testicular biopsies of males with infertility and to compare the results with data from other local and international studies.

**Materials and Methods:** All testicular biopsies from males with infertility received by the Pathology Department of King AbdulAziz University Hospital, Jeddah, in the period from January 2004 until May 2010 are reviewed and histopathologically classified into seven categories as follows : Normal spermatogenesis, hypospermatogenesis, germ cell maturation arrest (GCMA), Sertoli cell only syndrome, seminiferous tubule hyalinization, mixed and discordant patterns.

**Results:** One hundred testicular biopsies were identified in the computerized records of the Department of Pathology of King AbdulAziz University Hospital in the studied period. The age ranged from 22 to 70 years with a mean age of 24.5 years. The histopathological patterns were as follows: 14 (14%) cases were reported as normal spermatogenesis;(29, 29%) cases as hypospermatogesis; and 12 (12%) cases were reported as GCMA, mostly at the level of primary spermatocytes. The Sertoli cell only syndrome and the seminiferous tubule hyalinization categories were each reported in 16 cases (16%). Nine cases (9%) showed a mixed pattern. Discordant pattern was seen in 5 (5%) cases.

**Conclusion:** Our study showed that hypospermatogenesis is the commonest pattern in testicular biopsies taken from males with infertility in our region. This study supports the recommendation of bilateral testicular biopsies when investigating male infertility.

**Key Words:** Hypospermatogenesis, male infertility, testicular biopsy

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## INTRODUCTION

Infertility is defined as inability to conceive after 12 months of regular unprotected intercourse.[1] Although male infertility is a major cause of infertility among couples, contributing to more that half of all cases of infertility,[2,3] investigations of couple infertility have always concentrated on female pathological causes while male clinical conditions leading to infertility are still generally underdiagnosed and undertreated. The evaluation of the infertile male includes a thorough clinical history taking and physical examination, semen analysis, hormonal assay, and search for antisperm antibody. Additional tests include transrectal ultrasonography, vasography and testicular biopsy. The latter is particularly useful in cases of azoospermia or

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oligospermia and normal endocrine function.[4,5]

Testicular biopsies from infertile men with azoospermia or oligospermia usually show different pathological patterns. In this study, the pathological changes in testicular biopsies of all males with infertility are reviewed and results compared with local and international studies.

## MATERIALS AND METHODS

In this retrospective study, all testicular biopsies from males with infertility received by the Department of Pathology of King Abdul-Aziz University Hospital, Jeddah, were retrieved and slides and reports reviewed. Clinical data such as the age of the infertile male and sperm analysis result were extracted from the patient's medical records. Patients in this study had either unilateral or bilateral testicular biopsies under local anesthesia. Biopsy samples once taken were immediately placed into Bouins fixative and sent to the Department of Pathology, where all testicular biopsies were processed as routine, stained with hematoxylin and eosin (H and E) and examined histologically by light microscopy. The examined H and E slides were evaluated for the number and lumen size of seminiferous tubules in each biopsy, germ cell/Sertoli cell ratio, basement membrane thickening, presence or absence of seminiferous tubule hyalinization, the number of Leydig cells and presence of other findings such as tubular cells with carcinoma *in situ* (CIS), interstitial inflammation, or granuloma. The biopsies were examined for histological uniformity within the same testis and between right and left testes. The testicular biopsy was labeled "mixed" if more than one pattern was seen in the same biopsy (from the same side) and "discordant" if the right and left testicular biopsies showed different patterns.

All testicular biopsies were classified histologically[6] and tabulated [Table 1] into seven categories as follows.

Normal spermatogenesis: Tubules having thin basement membrane and tunica propria; as well as normal germinal epithelium showing orderly progression from spermatogonia to spermatocytes with groups of spermatids and mature spermatozoa.

**Table 1: Histopathological classification of testicular biopsies**

| Histological classification | No. | % |
|---|---|---|
| Normal spermatogenesis | 13 | 13 |
| Hypospermatogenesis | 29 | 29 |
| Germ cell maturation arrest | 12 | 12 |
| Sertoli cell only syndrome | 16 | 16 |
| Seminiferous tubule hyalinization | 16 | 16 |
| Mixed pattern | 9 | 9 |
| Discordant pattern | 5 | 5 |
| Total | 100 | 100 |

Hypospermatogenesis: The cellularity of germinal epithelium was reduced in general, all stages of germ cells (spermatogonia, spermatocytes, and spermatids) were present but reduced in number.

Germ cell maturation arrest (GCMA): The process of spermatogenesis was arrested at a specific cell stage mostly at the primary or secondary spermatocytes. This pattern differs from hypospermatogenesis by the absence of spermatids.

Sertoli cell only syndrome: In these cases, the tunica propria and basement membranes were not thickened appreciably, and the tubules were normal or slightly decreased in diameter, and contained only Sertoli cells but no other cells involved in spermatogenesis. The interstitium contained normal numbers of Leydig cells in most cases.

Seminiferous tubule hyalinization: The tubules were smaller in diameter with a much thickened basement membrane and tubular collagenization. The germinal epithelium was lost in these cases.

Mixed pattern: More than one pathological pattern was seen in the same testicular biopsy.

Discordant pattern: The right and left testicular biopsies showed different patterns.

The number and percentage of each pathological pattern was calculated.

## RESULTS

One hundred male patients with either unilateral or bilateral testicular biopsies were identified in the computerized records of the Department of Pathology of King AbdulAziz University Hospital in the period from January 2004 until May 2010. All cases were male patients who presented to the obstetrics and gynecology or urology clinic with infertility and had either azoospermia or oligospermia diagnosed on routine sperm analysis. Their age ranged from 22 to 70 years with a mean age of 24.5 years. Sixty-seven patients had unilateral and 33 patients had bilateral testicular biopsies.

The cases were classified according to the seven mentioned histopathological categories as presented in Table 1.

Nine biopsies (9%) showed a mixed pattern where more than one histopathological picture was seen in the same testicular biopsy [Table 2].

Discordant pattern where right and left testicular biopsies showed different patterns were found in 5 (5%) cases as shown in Table 3.

**Table 2: Testicular biopsies with mixed histopathological patterns**

| No. | Mixed histopathological pattern |
|---|---|
| 3 | Sertoli cell only syndrome + hypospermatogenesis |
| 1 | Sertoli cell only syndrome + germ cell arrest |
| 2 | Seminiferous tubule hyalinization + germ cell arrest |
| 2 | Seminiferous tubule hyalinization + hypospermatogenesis |
| 1 | Seminiferous tubule hyalinization + normal spermatogenesis |

**Table 3: Bilateral testicular biopsies with discordant histopathological patterns**

| No. | Discordant histopathological pattern |
|---|---|
| 1 | Seminiferous tubule hyalinization + normal spermatogenesis |
| 1 | Seminiferous tubule hyalinization + hypospermatogenesis |
| 1 | Seminiferous tubule hyalinization + germ cell arrest |
| 1 | Germ cell arrest + insufficient biopsy |
| 1 | Germ cell arrest + hypospermatogenesis |

No cases of tubular CIS, granuloma or orchitis were identified in the studies cases.

## DISCUSSION

The incidence of male infertility and the subsequent histological findings in testicular biopsies differ significantly from one part of the world to another due to several underlying etiological factors including social habits, genetic causes and environmental conditions such as underlying infections, chemicals, radiation and exposure to heat.[7,8]

Causes of male infertility are divided into three major categories: pretesticular, testicular and post-testicular causes.[9] The pretesticular causes of infertility include extragonadal etiologies such as endocrine disorders originating in hypothalamus, pituitary or adrenals, chronic illnesses including diabetes mellitus and hypertension, as well as certain medications. The testicular causes include defects in the process of spermatogenesis and post-testicular causes include obstructions of ducts draining the testes, related to trauma, surgery or mumps orchitis.[9] The distinction between post-testicular obstructive and pretesticular or testicular nonobstructive causes of male infertility is important since men with obstructive etiologies may have other costeffective options for treatment, such as microsurgical reconstruction of the reproductive tract.[10]

Testicular biopsy remains the key investigation for all testicular causes of infertility.[11] It is not the only parameter for determining the testicular histopathology pattern but apparently the strongest indicator to foresee the possibility of finding sperms in the testis for therapeutic sperm retrieval in assisted reproductive techniques.[12] In addition, testicular biopsy is important in the evaluation of men at risk for CIS or testicular cancer, such as those with idiopathic infertility, prior cryptorchidism, history of testicular neoplasia or the presence of suspicious clinical or radiological finding such as a nodule or microlithiasis.[11,12] Testicular biopsy can be performed under local or general anesthesia and compromise either a transcutaneous needle or open biopsies from one or more sites. Since the nature of various lesions may differ from one testis to another and heterogeneous pathological patterns are common, it is recommended to obtain bilateral testicular biopsies when studying male infertility[6]

In the present study, only 27 males had bilateral testicular biopsies taken for histopathological examination and five of these biopsies (18.5%) showed a discordant pattern.

Currently, there is no agreement on systemic reporting of testicular biopsies; terms used are not standardized and are often subjective with vague terms. The most commonly used histopathological classification of testicular biopsy depends on the presence and amount of spermatogenesis, maturation of germinal cells and presence of associated tubular atrophy, interstitial fibrosis and Leydig cell hyperplasia[6,12]

The present study results showed agreement with some international studies and discrepancies with others.

Our results showed low frequency of normal spermatogenesis which was identified only in 13(13%) cases out of the total number of cases studied, suggesting possible post-testicular obstructive etiologies. Few previous studies reported similar low incidences. Meinhard *et al.*[13] reported 5% for obstructive azoospermia; Haddad *et al.*,[14] in a study from Jordan, reported 11.2% for obstructive azoospermia; and Nagpal *et al.*[15] reported similar results (16%). On the other hand, others[10,16,17] reported much higher incidence of normal spermatogenesis. Ragab *et al.*[10] from Egypt reported normal spermatogenesis in 24% of cases. Wong *et al.*[9] recorded a similar result (25%). Also, Colgan *et al.*[18] reported obstructive azoospermia with normal histology in 20% of their cases. Brannen and Roth[19] reported a higher incidence of obstructive azoospermia (35%), and the same was reported by AlRayess *et al.*[16] (31%). Thomas[20] has conducted a study in Nigeria and reported an incidence of 38% for obstructive azoospermia with normal histology. Normal spermatogenesis suggests obstruction of some part of the ductal system such as varicocele and obstruction of rete testes.[6]

Hypospermatogenesis represented the most common finding (29 cases, 29%) in the present study. This finding is similar to another study from the same region of this country,[21] which reported an incidence of 25%,while it differs from two other local studies from the same country[16,22] which revealed a much lower incidence of hypospermatogenesis. Alrayes[16] from

Riyadh in his study of 230 testicular biopsies showed a 13% incidence of hypospermatogenesis, while Thomas and Jamal[22] from the western region of Saudi Arabia reported an incidence of 3.7% for hypospermatogenesis. Other international studies showed variable results. Haddad *et al*.[14] reported a high incidence for hypospermatogenesis (55.8%). Meinhard *et al*.[13] and Colgen *et al*.[18] also reported a high incidence of hypospermatogenesis of 46 and 49%, respectively. On the other hand, Thomas[20] and Wong *et al*.[9] reported 19and 23%, respectively. Jamali *et al*.[23] from Iran reported an incidence of 36.6% with a mean age of 32.4 years.

Hypospermatogenesis is defined as an equivalent decrease in the numbers of spermatogonia and primary spermatids. In other words, all elements of spermatogenesis are present but decreased in number.[6] Clinically, hypospermatogenesis can be associated with hormonal dysregulation, congenital germ cell deficiency, androgen insensitivity, chemical exposure and exposure to heat and radiation.[1] Discrepancy between different studies can be explained by the different criteria used in selecting patients for biopsies. Some centers consider testicular biopsies only for patients with azoospermia, while others perform testicular biopsy for patients with either azoospermia or oligospermia. In the current study, testicular biopsy was performed for patients with either oligospermia or azoospermia.

GCMA is characterized by a block in the maturation to spermatids and therefore no mature sperms are present. Affected tubules tend to arrest at either the primary spermatocyte or spermatogonia stage. Clinically, there are many underlying etiologies for GCMA. The arrest may be caused by genetics or by secondary influences.[10] Genetic etiologies include trisomy, balanced autosomal anomalies (translocations, inversions) or deletions in the Y chromosome (Yq11).[24,25] Secondary causes include excessive alcohol or other toxic agent consumption, chronic marijuana use, cytotoxic chemotherapy and hypogonadotrophic hypogonadism.[6]

The incidence of GCMA in the present study was 12% (12 cases). The incidence was similar to that reported in several local studies[16,22] but much lower than the international figures.[10,15,17,23] In a study by Rashed *et al*.[10] from Egypt, the incidence of the GCMA was 28%, while Thomas[20] from Nigeria and Jamal[21] from Saudi Arabia reported low incidences of 5 and 7%, respectively. A very low incidence of GCMA (1.7%) was reported by Haddad *et al*. [14]

Sixteen cases (16%) of Sertoli cell only syndromes were identified in the present study. This finding correlates well with three other studies[15,21,23] in which similar figures were reported. Jamal[21] from the western region of Saudi Arabia reported an incidence of 16.5% while a second study from the same region of the country[22] reported an incidence of 27.2%. A similar high incidence (39% ) was also reported by Alrayes[16] from Riyadh , Saudi Arabia. The difference in the incidence of Sertoli cell only syndrome between local studies as well as between our study and several international studies cannot be totally explained. The term Sertoli cell only syndrome should only be applied to a universal pattern wherein no germ cells are seen in any profile. Sertoli cell only syndrome is an irreversible change that can be associated with many underlying conditions. These include cryptorchid testis, orchitis, post radiation or chemotherapy, estrogen or androgen therapy and as a consequence of chronic hepatopathology.[6] Recently, structural abnormalities of the Y chromosome, especially deletions of a gene called human azoospermia factor (AZF) located in the long arm of chromosome Y, have been blamed as the underlying cause for disturbed spermatogenesis and azoospermia.[24,25]

Seminiferous tubule hyalinization was reported in 16 cases (16%). Similar results were obtained by Thomas and Jamal,[22] Alsamawi,[17] and Jamali and Hairiri,[23] while much lower incidence was reported by Alrayes,[16] Nagpal[15] and Rashed and Ragab.[10]

Jamal[21] from the same region of Saudi Arabia and Thomas[20] from Nigeria reported higher incidences of 24and 22.4%, respectively. Although the reason behind this discrepancy in the incidence of seminiferous tubule hyalinization is not known, biopsy selection criterion between different urologists in the same center and in between various centers is one reason. Jay Thomas[20] in her study from Nigeria suggested that previous inflammatory processes such as previous orchitis may play a role in the causation of this entity.

The incidence of mixed patterns within the same biopsy and the presence of discordant patterns in right and left testes are common.[12] In the present study, nine mixed cases (9%) mainly of tubule hyalinization or Sertoli cell only syndrome mixed with GCMA or hypospermatogenesis was detected. In addition, five discordant cases (5%) with different patterns identified in right and left testes were found. McLachlan *et al*.[12] studied 534 consecutive men referred for bilateral testicular biopsies for investigation of infertility and demonstrated the relative rarity of pure phenotypes and the high frequency of hypospermatogenesis and mixed patterns. These findings support the use of bilateral testicular biopsies for comprehensive evaluation of male infertility and indicate the great importance of meticulous pathological evaluation and reporting of all observed patterns.

In addition, there is an important therapeutic benefit in performing bilateral testicular biopsies when considering intracytoplasmic sperm injection (ICSI) in the management

of infertile couples with male infertility which depends on the sperm retrieval and subsequent injection into a female ovum. The absence of sperms in one testis in such cases does not rule out their presence in the other.

The difference between the present study and some local and international studies is not well understood.However, previous studies from the same region of Saudi Arabia[16,22] as well as other Middle Eastern countries such as Egypt[10] suggested the influence of environmental factors, sociocultural habits and consanguineous marriages. This study stresses the need for large-scale multicenter study to reach a solid conclusion in this delicate and important issue of male infertility.

Recent studies show that quantitative assessment of human spermatogenesis is possible and can be beneficial.The 4D4 immunolabeling of testicular biopsies allows the establishment of new characteristics of normal and disturbed human spermatogenesis and the study of germ cell sloughing.[26] Recently, fine needle aspiration (FNA) has been introduced as a simple, lowcost and lowrisk procedure that is adequate for differentiating between obstructive and non-obstructive azoospermia and for assessing different spermatogenic defects as it shows good agreement with open biopsy data.[10,27] FNA is also used for sperm retrieval with minimal cost and morbidity. Unfortunately, this procedure is not used commonly in our region most probably due to the poor expertise in performing or interpreting its results.

## CONCLUSION

This study outlines the different patterns of testicular biopsy in cases of male infertility encountered in our region and identifies hypospermatogenesis as the most common pattern of spermatogenic defect among the different studied patterns. This study has also shed some light on the possible underlying etiologies for such defects in spermatogenesis and stresses the need for bilateral testicular biopsies as well as the need for meticulous pathological examination of all seminiferous tubules in order to identify mixed and discordant patterns.

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