Testicular morphologies in subjects evaluated for infertility in Brunei Darussalam

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ABSTRACT

Introduction: Infertility is a major problem in the present society and treatment is expensive. Men and women are equally responsible. Although treatment of the female infertility has advanced considerably, there is little progress in the treatment of male infertility. Three major groups of tests, namely hormonal assays, specialised andrological tests such as semen analysis and testicular biopsy are used to investigate male infertility. However, hormonal assay and semen analysis may not provide sufficient information about the condition of the testes. Therefore, testicular biopsy is crucial for proper assessment with both diagnostic and prognostic importance. This study retrospectively assessed the morphological patterns of testicular biopsies of men who had been investigated for infertility in Brunei Darussalam. **Material and Methods:** All testicular biopsies performed for the investigation of male infertility over a 10 years period (2001–2010) were included. These cases were retrieved from the laboratory information system RIPAS Hospital, Brunei Darussalam. Relevant slides were retrieved from the histological laboratory archives and reviewed in a blinded fashion. **Results:** There were 80 testicular biopsies included in this study with age range of between 21 and 48 years. A normal histological pattern was seen in 10 patients (12.6%). Of those with abnormalities, 21 (26.2%) showed maturation arrest, nine (11.3%) showed marked hypoplasia, 10 (12.5%) showed Sertoli cells only and four (5%) had sperm granuloma. There were four (5%) cases of testicular tuberculosis, seven (8.7%) with non-specific inflammation, one (1.2%) with a viral inclusion in the tubules and one (1.2%) with in-situ carcinoma. **Conclusion:** Our study shows that infertility can be due to various causes in man and testicular biopsy should be an integral part of investigation.

Keywords: Male infertility, testicular biopsy, azoospermia, Sertoli Cell Only

INTRODUCTION

Infertility is a global problem, which causes anxiety amongst affected couples and with both males and females sharing equal responsibility for the problem. Treatment is very expensive and outcomes are often not satisfactory. There are many causes of male infertility and routine hormonal and semen analysis do not always reveal the extent of the problem. In 1940, testicular biopsy was introduced as an investigation for male infertility. [1] Earlier on, testicular biopsies for the investigation of azoospermia. [2] In 1973, Meinhard *et al.* advocated testicular biopsy

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for complete assessment of the testes in men evaluated for infertility. [3] The present day treatment of infertility with assisted reproductive technology needs further evaluation of the testes in order to predict recovery of the spermatids by testicular sperm extraction as part of treatment of infertile couples. A previous study of infertility based on semen analysis in Brunei Darussalam showed that 4.6% of 1,242 subjects who had semen analyses were azoospermic. [4] Some of the causes of azoospermia cannot be detected by semen analysis and requires analyses of the testes. This study evaluates the various morphological patterns of testicular biopsies from men with infertility.

MATERIALS AND METHODS

This retrospective study was conducted on all testicular biopsies performed for the investigation of male infertility during 2001–2010. These were bilateral testicular biopsies performed on patients having hypospermatogenesis or azoospermia. A total 82 cases were retrieved through the Laboratory Information System (LIS) RIPAS Hospital, Brunei Darussalam. All the biopsies were performed by open access method and fixed in Bouin's fixative. All the biopsies were processed as routine 5µ cut sections stained with Hematoxylin and Eosin (H&E) and Martius yellow Scarlet Blue (MSB) stain and examined histologically by light microscopy. Relevant slides were retrieved from the archives maintained by the laboratory. The authors reviewed and classified all biopsies blinded to the clinical information and any discordant results were reviewed and discussed until agreements were reached. The biopsies were diagnosed as – normal, hypospermatogenesis, maturation arrest, sertoli cells only, atrophy, infections, and mixed patterns. [5] There were altogether 82 biopsies performed as part of investigations for infertility. Of these, two specimens were sent in formalin fixative and were not suitable for analysis and excluded from the study. The number and percentage of each pathological pattern was calculated.

RESULTS

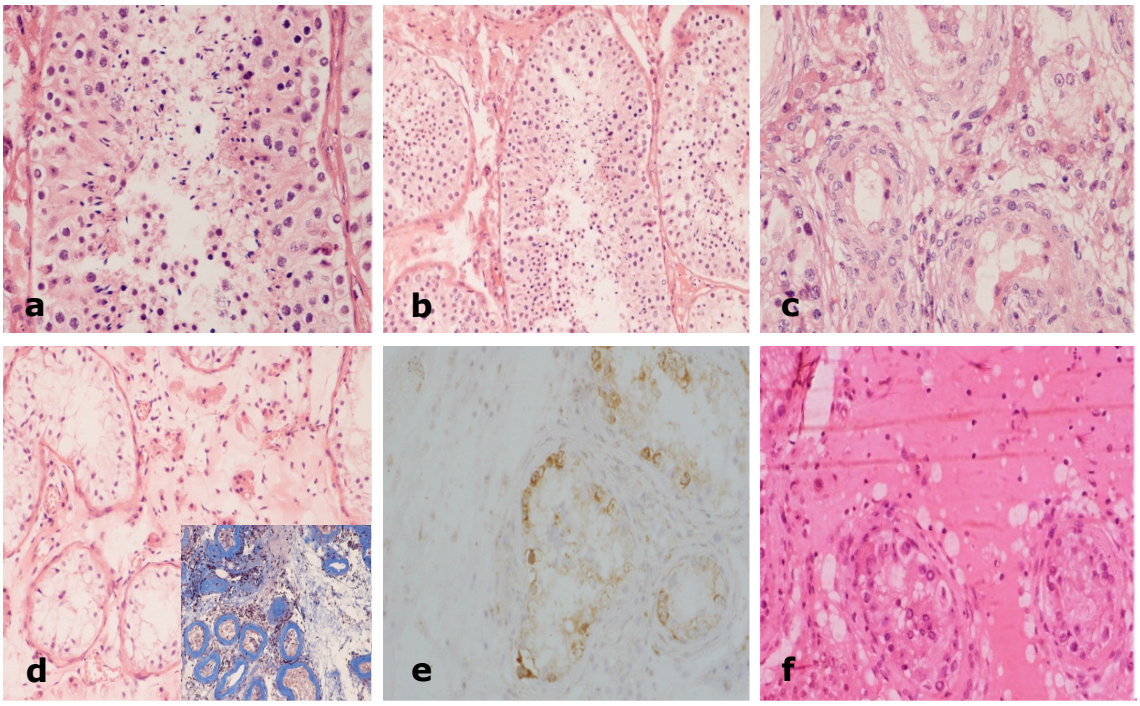
A total of 80 cases received over a 10 year period were studied. The age of patients ranged between 21-50 years. The histological diagnoses are shown in Table 1.

***Normal*:** 12.5% of the biopsies showed normal spermatogenesis. All the tubules were uniform in size and diameter measured approximately 200µm. Orderly progression of spermatogonia to spermatocytes were seen. Sertoli cells were compressed between germinal cells and not easily identified. The basement membrane was thin and uniform. A few Leydig cells could be seen in the interstitium.

Maturation arrest: In maturation arrest, the tubular diameters were normal. Spermatogonia were present and normal. Maturation was

Table 1: Histological diagnosis of patients.

Histological findings	Number (%)
Normal	10 (12.5)
Maturation arrest	21 (26.2)
Marked hypoplasia	9 (11.3)
Sertoli cells only	10 (12.5)
Tuberculosis	4 (5)
Sperm granuloma	4 (5)
Non-specific infection	7 (8.8)
Neoplasia (ITGCN)	1 (1.3)
Obstruction	14 (17.5)



Figs. 1: a) Normal case: normal basement membrane tubules line by spermatogonia, spermatocytes and spermatids (H&E stain). Normal basement membrane tubules line by spermatogonia, and spermatocytes. Only few spermatids are seen (H&E stain), b) Maturation arrest (H&E stain), c) Hypoplasia of the tubules, d) Sertoli Cell Only (insert showing thickened basement membrane), e) Immunohistochemistry stain showing the presence of Herpes Simplex (HSV) 11 antibody, and f) HSV 11 infection with lymphocytic infiltration of the stroma (H&E stain).

nia were present and normal. Maturation was arrested in the primary or secondary spermatocyte stage. In some cases there were no or only a few spermatids could be identified.

Hypoplasia: Tubules were of normal size and the germ cell epithelium was arranged in orderly pattern. All stages of germ cell epithelium; spermatogonia, spermatocytes, and spermatids were identified, although the cellularities were markedly reduced. Sertoli cells were clearly seen. Occasional scanty spermatozoa were identified.

***Sertoli Cells Only*:** Tubules were of small size. With marked thickening of the basement membrane. Some tubules were hyalinised. Germinal epithelium was not seen. Leydig cells were seen in clusters.

DISCUSSION

Testicular biopsy is an important in the investigation of male infertility. Clinical examination, hormonal assay and semen analysis may yield insufficient information to plan appropriate management. Hence, testicular biopsies are required in some cases.

There are many parameters that need to be taken into account when investigating patients with infertility. Jequier *et al*. reported that in Sertoli cell only syndrome, the testicular size is not markedly reduced. [6] However, testicular size may provide indication of the

severity of the disease process, but this is not precise in the intermediate stage. [7] In our study, all the testes biopsied were recorded as of normal size except in five cases. These five cases were reported as small. However these were subjective and no measurements were recorded. Therefore, clinical diagnosis based on testicular and other parameters may not provide adequate information and the underlying aetiology without testicular biopsy.

In our study, 10 cases (12%) were categorised as histologically normal. The tubules were regular and uniform in size with thin basement membrane and tunica propria. The germinal epithelium was normal and showed orderly progression from spermatogonia to spermatocytes with groups of spermatids and mature spermatozoa. Normal tubules in an azoopermic patient typically suggest post testicular obstruction. Several studies also reported similar low incidence of normal testicular biopsy in patients investigated for infertility. Nagpal *et al*. [8] reported 16%, Hadad *et al*. [9] in a study from Jordan reported 11.2% and Meinhard *et al*. [3] reported 5%. Several studies had reported high incidence of normal testicular biopsy. Ragab *et al*. [10] from Egypt reported high incidence of normal spermatogenesis (24%) in azoospermic patients, Wong *et al*. [5] reported 25% cases, Colgen *et al*. [11] in 20% of cases and Brannen and Roth [12] reported normal spermatogenesis in azoospermia in 35% of their cases. Thomas reported highest incidence (38%) in Nigeria. [13] Infertility in these cases may be due to the obstruction to some part of the ductal system.

Four of our cases (14%) showed sloughing of the epithelium with varying stages of spermatogenesis, including mature sperms without orderly arrangement. In addition, there were four cases of tuberculosis (5%), four cases of sperm granuloma (5%) and seven cases of non-specific infection (8.75%). One patient had evidence of infection with *Herpes simplex virus* 11 with nuclear inclusions, which stained positively with antibodies. Azoospermia and hypospermia in these cases may be partly due to obstruction and partly due to direct damage of the functioning tubules.

There were 21 cases (26.2%) of maturation arrest in our study. Morphologically there is interruption of normal germ cell maturation. The tubules tend to arrest at spermatogonia or spermatocyte stage. The cause for maturation arrest is diverse either genetically mediated or due to secondary influences. Genetic causes include trisomy, balanced autosomal abnormalities and Y chromosome deletion. Secondary causes include excessive alcohol intake, cytotoxic chemotherapy, habitual drugs specially marijuana use and other toxic agent consumption. Also contributing to maturation arrest is excessive heat and general diseases of the liver and kidney. Some patients with sickle cell anaemia have had maturation arrest.

Incidence of maturation arrest varies in different countries and different institutions even from the same country. The rates reported from Saudi Arabia were between 7% and 12%. [14, 15] One study from Nigeria reported a low rate of 5% [13] and a study from Egypt reported high rate of 28%. [17] Some of the international studies reported higher figures similar to our study.

There were nine cases of marked hypoplasia (hypo-spermatogenesis), tubular pattern is normal. Germinal epithelium in all stages is present but reduced in number. Clinically hypo-spermatogenesis is associated with congenital germ cell deficiency, androgen insensitivity, exposure to chemicals, extremes of heat and radiation. Our incidence is similar to other studies.

There were 10 cases (12.5%) of Sertoli cells only syndrome in our study. Tubules were uniformly well preserved and lined only by Sertoli cells. Some cases showed hyperplasia of the Leydig cells. There were no spermatogonia or spermatocytes. One case showed hyalinisation of the tubules with thick basement membrane (major tubular atrophy), suggestive of Klinefelter syndrome. Subsequent chromosomal analysis confirmed the diagnosis. The incidence of Sertoli cells only syndrome varies in different countries. Rasheed *et al*. [17] from Egypt reported a high incidence of 34%. Studies from Saudi Arabia reported varying incidence; 39% in Riyadh [18], 16.5% from western region, [15] whilst a second study by the same group reported 27.2%. In contrast, other countries have reported low incidence. One study from Nigeria reported a rate of 9% [13] and older studies from the United States reported rates of between 8% and 12.5%. [5, 11, 12] Our results are comparable to countries with low incidence.

There are many causes of Sertoli cell only syndrome. Cryptorchidism, exposure to cytotoxic drugs or radiation exposure are important secondary causes. However, in many cases, no aetiological factor are detected. The absence of germ cells is believed to due to effects of deleterious factors during foetal life. [19] Carrara *et al.* reported that consanguinity may cause Sertoli cell only syndrome and concluded that this disorder may be due to recessive gene mutation. [16] The genetic region controlling the human spermatogenesis, the Human azospermic factor (AZF) localised to the long arm of the Y chromosome have also been identified. [16] Azoospermia and high incidence of deranged spermatogenesis have been reported in men who had deletions corresponding to the AZF region.

There were seven cases of non-specific orchitis (8.75%) and four cases of tuberculosis (5%) in our study. In the non-specific group, one had viral inclusion which stained positive with HSV 11 viral antibodies, suggesting a HSV infection. Studies have also reported viral inclusion bodies in approximately 6% of cases. [3, 17] However higher incidence of 23% [13] and 28.4% [9] have been reported.

In our study, one patient had in-situ neoplasia. This patient had cryptorchidism, a condition associated with malignant transformation corrected at the age of 12.

In conclusion, distinction between obstructive and non–obstructive azoospermia is important as the treatments are different. Men with obstructive azoospermia will have a remedial option, such as surgical correction of the obstruction or sperm retrieval for intracytoplasmic sperm injection for in-vitro fertilisation. Treatment for non-obstructive azoospermia is still very limited. This study confirmed that the testicular biopsy is an important tool and provides information on the aetiological factor as well as essential prognostic information.

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