HISTOPATHOLOGICAL FINDINGS IN THE TESTICULAR TISSUES OF PATIENTS WITH AZOOSPERMIA ATTENDING AMINU KANO TEACHING HOSPITAL KANO, NORTH-WESTERN NIGERIA

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ABSTRACT
Background: Infertility is a common problem affecting young couples. It is estimated that about 50-80 million couples worldwide experience some form of infertility resulting in considerable psychological and social distress to them. Male factor infertility accounts for about 30-50% of couple infertility depending on geographic locality.
Aim: This ten year retrospective histological review was carried out to determine the, frequency, age distribution and histologic patterns of the testicular causes of male infertility seen in patients that presented with infertility and azoospermia at Aminu Kano Teaching Hospital, Kano from January, 2009 to December, 2018.
Methodology: This is a ten year retrospective histopathological study of one hundred and ninety (190) testicular biopsies done on the indication of azoospermia and infertility selected based on WHO criteria. Archival tissue blocks were freshly cut with a microtome at 4 micrometer thickness and the sections were stained using Haematoxylin and Eosin, Histochemistry (Periodic Acid Schiff) and Immunohistochemistry (Vimentin).
Results: Forty-nine (29%) showed normal morphology and was the most frequent histologic finding and pattern representing blockage along the draining ducts of the testes followed by forty-five (26.5%) cases showing variable degree of testicular atrophy. Maturation arrest, Germ cell aplasia, Hypospermatogenesis and Adenomatoid tumour representing 17.2%, 16.0%, 10.1% and 1.2% respectively were the other morphologic findings. One hundred and fifteen (60.5%) of these testicular lesions occurred in the third and fourth decades of life presumably because most marriages occur in that age group and a year after marriage without conception qualifies one for infertility and investigation.
Conclusion: Obstruction, maturation arrest and hypospermatogenesis are potentially curable conditions, therefore profiling and documentation of the testicular morphology in the azoospermic patients is essential for the institution of appropriate management.
Keywords: Infertility, azoospermia, testis, AKTH, Kano.

INTRODUCTION
The World Health Organization (WHO, 2000) defines infertility as the inability of a sexually active, noncontracepting couple to achieve pregnancy in one year. It can also be considered as the inability of the couple to conceive within two years of exposure to the risk of pregnancy (WHO, 2001). Rowe *et al,* (1993) defines infertility as the failure of conception after at least 12 months of unprotected intercourse. An infertile or subfertile male is defined as a man who fails to produce a child after trying for one year but whose female partner is fertile (Ming *et al*., 2012). It is clear that infertility is a common problem affecting young couples, and equally clear that it results in considerable distress for those couples affected. The feelings experienced by infertile couples encompass anger, depression, anguish, denial, guilt, shame, inadequacy, shock, isolation and embarrassment (Wright *et al.,* 1991).

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It is estimated that 50-80 million couples worldwide experience some form of infertility and 20-35 million of these couples are in Africa (WHO, 1991). In the United States of America, an estimated 5 million men are affected (Schlegel, 2009). Whereas in parts of Sudan and Cameroon, up to 40% of marriages are infertile (Kleinman & Senanayake, 1978). Male factor infertility accounts for 30-50% of couple infertility in Nigeria (Patrick & Abiodun, 2015).

Broadly, the causal factors for male infertility can be divided into genetic and non-genetic (Makker *et al.,* 2009; Sharma, 2017). The genetic factors include various kinds of chromosomal abnormalities like deletions, inversions, mutation aneuploidy, and translocation and of these, translocation is the most common chromosomal abnormality (Faed *et al.,* 2012). The non-genetic include exposure to heavy metals like Lead, Cadmium and Mercury, Pesticides and Other Polychlorinated Hydrocarbons and free radicals. Others include life style, advanced age, obesity, smoking, use of mobile phones and use of certain recreational drugs (Sharma, 2017).

The causes of male infertility are also classified into pre-testicular, testicular and post-testicular (Dimitriadis *et al*., 2017). The pre-testicular causes are extra-gonadal endocrine disorders usually originating in the pituitary or adrenal gland. The testicular causes are primary diseases of the testes and little treatment is available at the present time. The post-testicular causes consist mainly of obstructions of the ducts leading away from the testes. These obstructions may be congenital, post- inflammatory or post-surgical and include the Young syndrome in which the obstructive azoospermia is combined with chronic sinopulmonary infections. These obstructions generally have little or no adverse effect on spermatogenesis (Rosai & Ackerman, 2015).

The evaluation of the infertile male includes a thorough clinical history and examination, semen analysis, quantitation of leucocytes in semen and search for antisperm antibodies. Additional tests performed in selected cases are transrectal Ultrasonography, Venography and testicular biopsy (Cerilli *et al*, 2010).

Biopsy specimens from infertile men with azoospermia defined as total lack of spermatozoa usually show one of the following conditions (Nelson, 1961): Normal spermatogenesis which suggests an obstructive azoospermia and implies the bilateral obstruction or absence of some part of the duct system; Spermatocytic arrest, characterized by the halt of maturation sequence, usually at the stage of primary spermatocytes; Hypospermatogenesis which is the reduction of the population of germ cells and a poor order of spermatogenesis with occasional tubular hyalinization; Germ cell aplasia (Sertoli cell-only syndrome) where the tubules are populated by Sertoli cells cytoplasm of which may contain Charcot-Bottcher crystalloids, representing bundles of filaments which are immunohistochemically positive for vimentin (Rosai & Ackermann, 2015) and; Generalized fibrosis and Atrophy. This is characterized by testicular architectural distortion by fibrosis and variable tubular atrophy and hyalinosis (Rosai & Ackermann, 2015).

MATERIALS AND METHODS

Archival tissue blocks of testicular biopsies from infertile males by WHO definition were retrieved from the department of Histopathology, Bayero University/ Aminu Kano Teaching Hospital Kano. Three hundred and thirty eight (338) archival paraffin embedded tissue blocks of right and left testicular tissues of infertile males processed over a period of ten years (January, 2009 to December, 2018) were retrieved from the archives of the department. Fresh sections were cut at 4 micrometer and stained with Haematoxylin and Eosin (H&E). Where applicable, a special stain Periodic Acid Schiff (PAS) and Immunohistochemical stain Vimentin for the demonstration of Sertoli cells were also used.

RESULTS

A total of three hundred and sixty eight (368) testicular biopsies were received and analyzed at the department of Pathology of Bayero University/ Aminu Kano Teaching Hospital Kano over the ten year period covered by this retrospective study. The various indications for the biopsies included intersex and other congenital abnormalities, undescended testis, hydrocoele, inflammatory and infective conditions and tumours.

One hundred and ninety (190) biopsies were done on the indication of either primary or secondary infertility but only one hundred and sixty nine (169) constituting 88.9% were bilateral and accompanied by seminal fluid analysis (SFA) result that indicated azoospermia thus qualifying for the study.

Table 1 depicts the frequency of histomorphologic alterations seen in the testes of the infertile males. The most frequent morphology was normal preserved histology representing 29% (49) of the cases followed by variable degree of atrophy accounting for 26.5% (45) of all the cases. Maturation arrest at the level of primary or secondary spermatocytes was the third most frequent morphology (17.2%) followed by Germ cell aplasia and Hypospermatogenesis accounting for 16% and 10.1% respectively.

Two cases of adenomatoid odontogenic tumour of the testes presenting with primary infertility were seen and noted in their 4th and 5th decades of life.

A case of primary infertility in a 29 year old adult male was reviewed. The testicular tissue showed partial effacement of its native architecture by schistosoma ova induced granulomatous inflammation but with few preserved tubules containing all the spermatogenic elements. There was also extension of the granulomatous inflammation in to the epididymis accompanied by marked fibrosis suggesting an obstructive azoospermia as it was reclassified to Normal spermatogenesis.

Table 1: Frequency of Histomorphologic Findings Seen in the Testes of the Infertile Males

Finding	Number (n)	Percentage (%)
Obstruction/ Normal	49	29.0
Testicular Atrophy	45	26.5
Spermatocytic Arrest	29	17.2
Germ Cell Aplasia	27	16.0
Hypospermatogenesis	17	10.1
Adenomatoid Tumour	2	1.2
Total	169	100%

The most frequent histologic pattern in the testes were normal morphology and normal spermatogenesis. These constituted 29.0% of all the cases and are characterized by regularly arranged seminiferous tubules bordered by basement membranes. The lumina are populated by Sertoli cells and spermatogonia arranged towards the basement membrane followed by primary and secondary spermatocytes, spermatids and spermatozoa with the latter concentrated at the centre of the lumen and in large numbers. The interstitial connective tissue is scanty and supports few clusters of Leydig cells (Plate I).

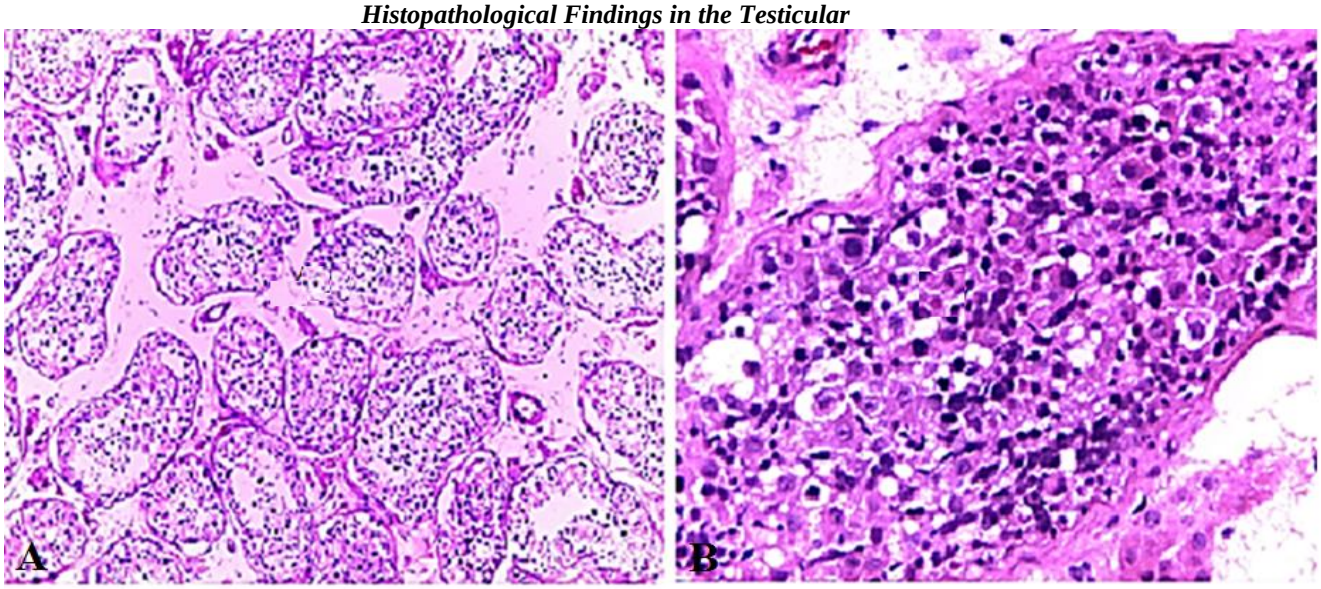

Plate I: (A) Seminiferous Tubules containing all Germ Cells Including Mature Spermatozoa. H&E X 100. (B) Redistribution, Disorganization and Sloughing of the Germ Cells. (H&E X 400).

Testicular atrophy represented the second most common histologic finding seen in the testicular tissues of the infertile males with azoospermia representing 26.5% of all the cases. Testicular atrophy is characterized by thickening of tubular basement membranes with focal or complete hyalinization of the seminiferous tubules depending on the severity of the atrophy (Plate IIA).
The thickening of the basement membrane is highlighted by positive Periodic Acid Schiff stain. (Plate IIB).

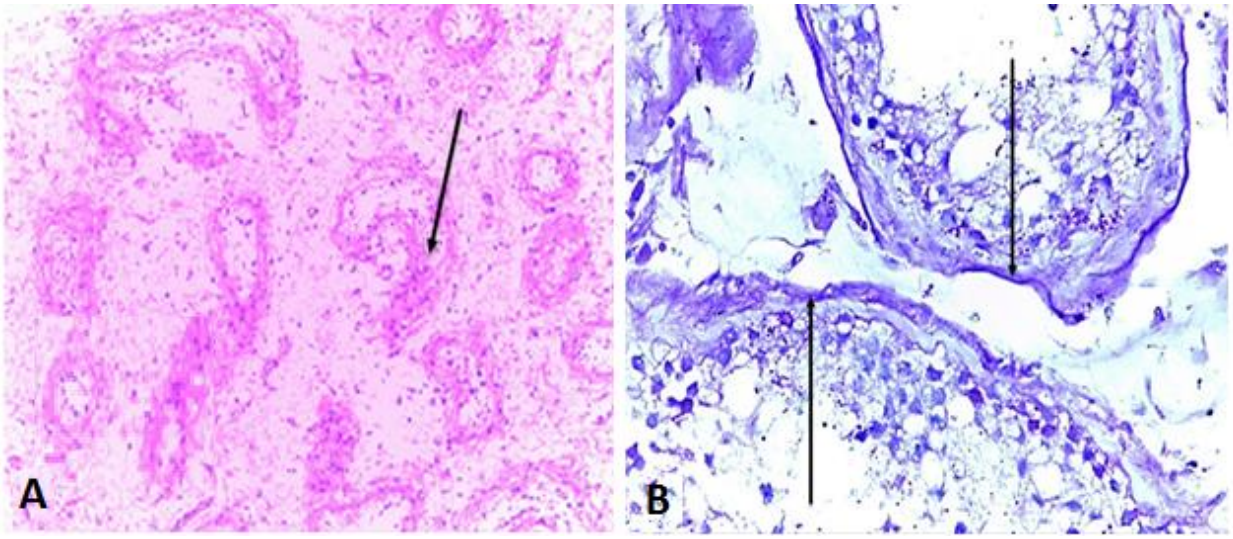

Plate II: (A) Small Atrophic Tubules, Thickened Basement Membranes and Fibrotic Interstitium (H&E X 400). (B) Periodic Acid Schiff (PAS) highlighting Thickening of Basement Membrane (Arrow) (PAS X400)

The third most frequent histologic pattern was variable degree of spermatocytic arrest. This accounted for 29 cases representing 17.2%. Arrest may occur at the level of primary to secondary spermatocytes or spermatids to spermatozoa referred to as spermiogenic maturation arrest. These are morphologically characterized by absent spermatozoa in the tubules. (Plate IIIA, B).

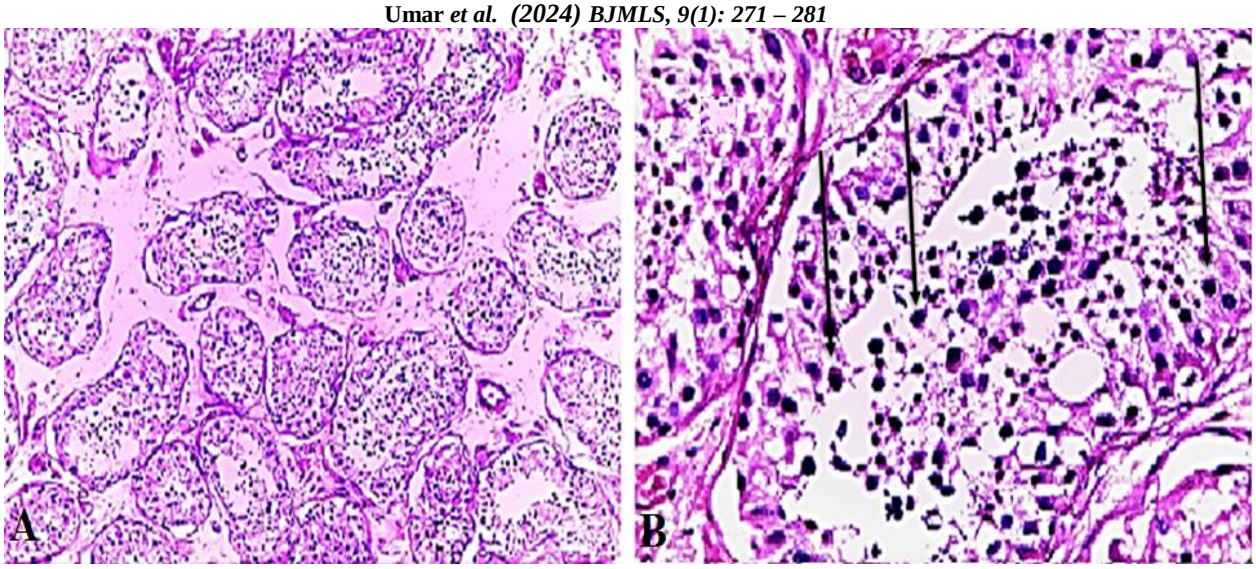

Plate III: Spermatocytic Arrest at the Level of Secondary Spermatocytes (Arrows). H&E X100 (A), X400 (B).

Germ cell aplasia or Sertoli cell-only syndrome constituted 27 cases representing 16.0%. It is characterized by seminiferous tubules populated by Sertoli cells only; no germ cells present (Plate IVA). Sertoli cells contain filaments in their cytoplasm which stain positive for immunohistochemical stain Vimentin (Plate IVB).

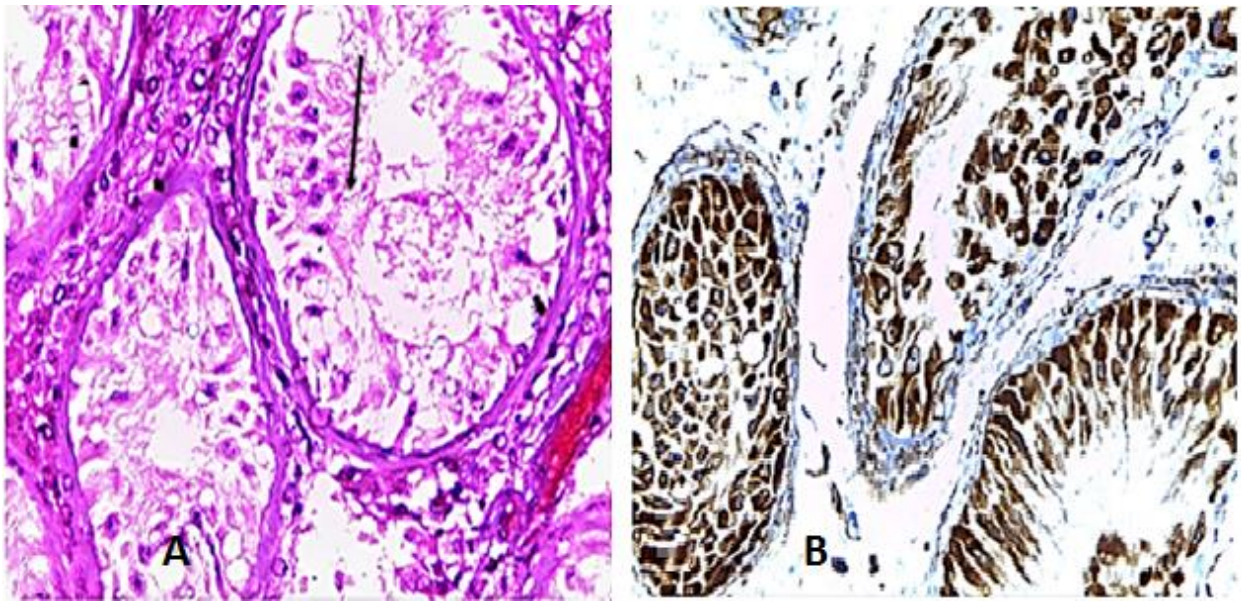

Plate IV: A- Germ Cell Aplasia, all the Seminiferous Tubules Contain Sertoli Cells Only (H&E X400). B- Immunohistochemistry (IHC – Vimentin): Sertoli Cells Showing Diffuse and Intense Stain (+++). (H&E X400)

Hypospermatogenesis is the reduction in number of germ cells in the seminiferous tubules. Despite the presence of small numbers of spermatids and spermatozoa, their SFA result were presented as azoospermic. They may well represent a case of obstruction but hypospermatogenesis is a recognized morphologic entity responsible for infertility. There were 17 (10.1%) cases of hypospermatogenesis (Plate VA, B).

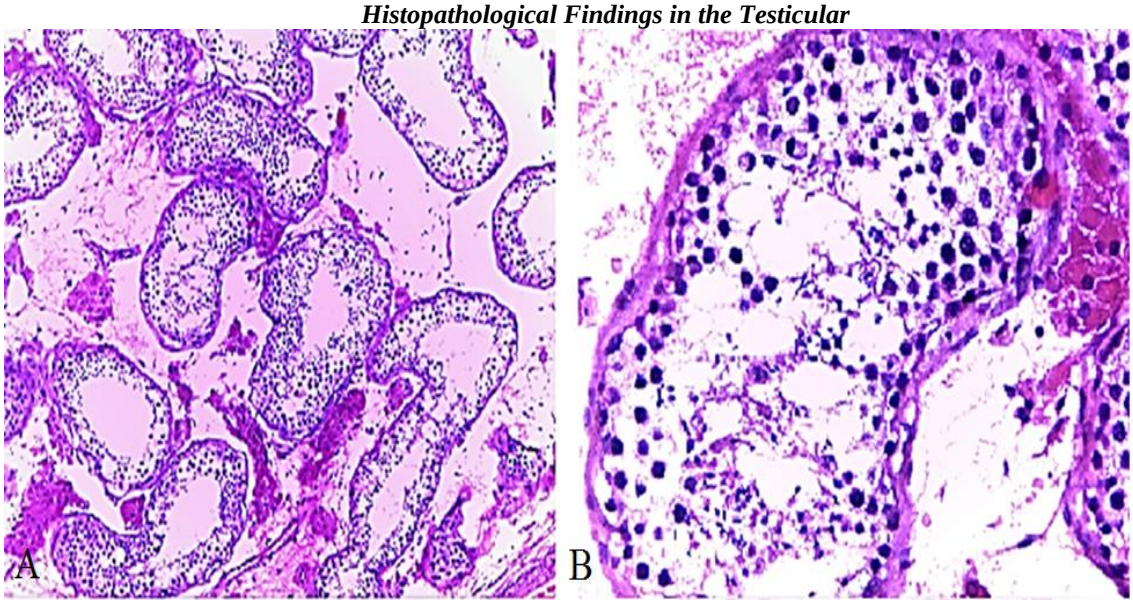

Plate V: Hypospermatogenesis - Reduced Number of Germ Cells with Marked Paucity of the Mature Forms (H&E X 100, 400)

Two cases of germ cell aplasia (Sertoli cell-only syndrome, VIA) were reassigned to hypospermatogenesis (VIB) after taking deeper sections of the tissue blocks and immunostaining with vimentin which showed negative staining pattern (Plate VIC).

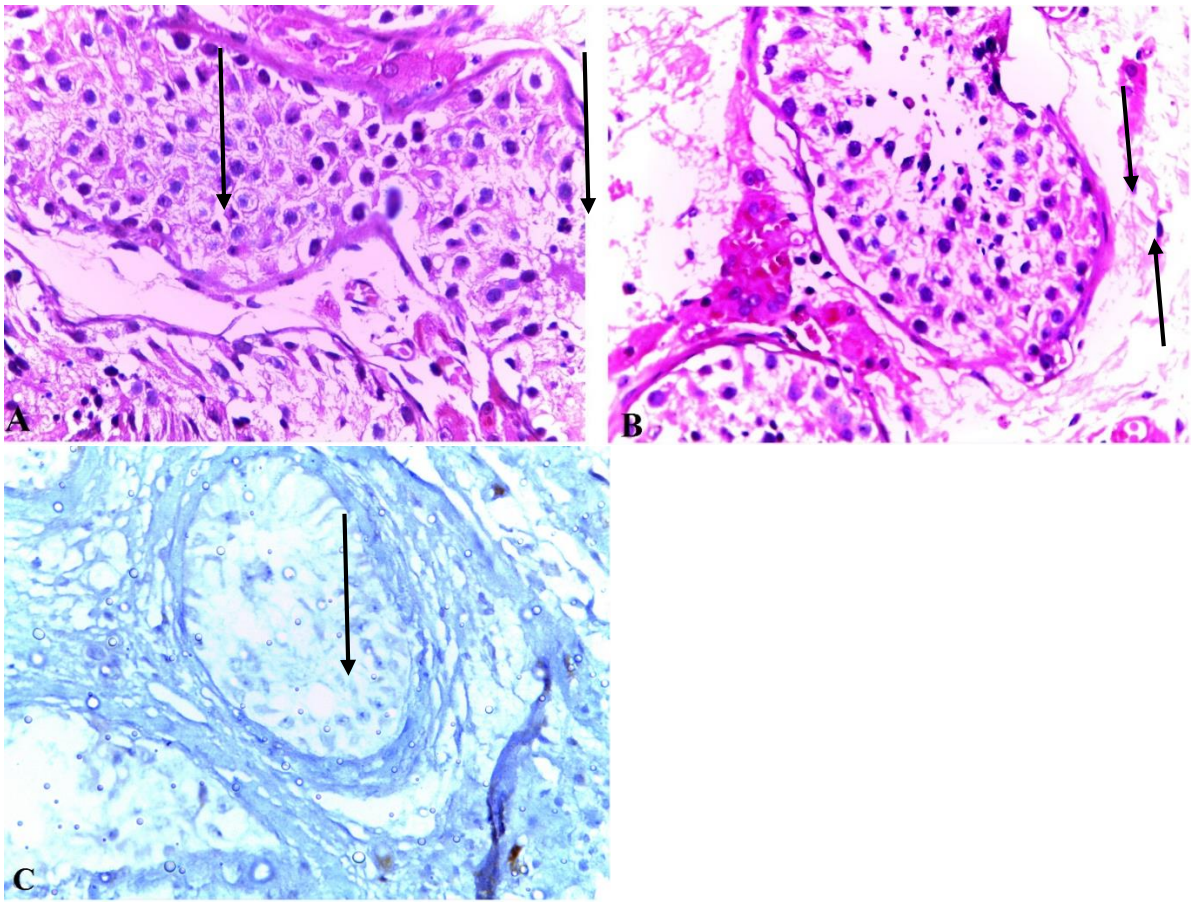

Plate VI: (A) Some Tubules populated mostly by Sertoli Cells with fewer Spermatocytes and Spermatids (Arrows) (H&E X 400). (B) Same Testis showing many Tubules containing all the Germ Cells with slight reduction in the number of Spermatozoa (Arrows) (H&E X 400). (C) Negative Immunohistochemical Stain with Vimentin (Arrow) (H&E X 400)

Three cases of testicular atrophy were also reassigned to spermatocytic arrest after reembedding the tissue block. The initial sections showed fibrosis and hyalinization of the tubules with few germ cells (VIIA) but the reembedded section showed most of the tubules containing the germ cells to the level of secondary spermatocytes. (Plate VIIB).

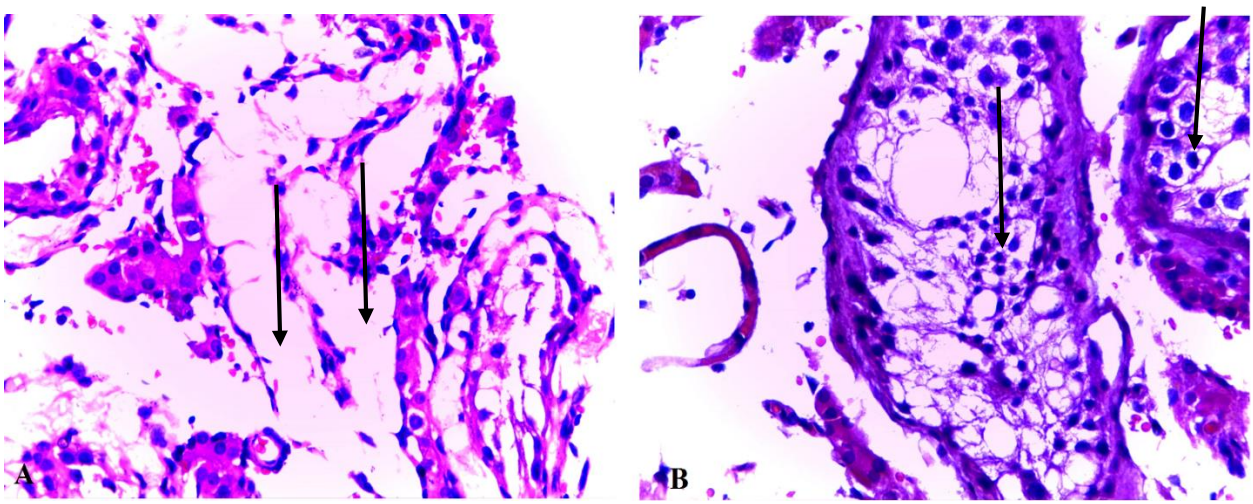

Plate VII: (A) Testicular Atrophy showing some Hyalinized Tubules (Arrows). (B) Same Testis reembedded and showing spermatocytic arrest at Secondary Spermatocyte level (Arrows) (H&E X 400).

Adenomatoid tumour is a benign paratesticular tumour derived from the mesothelial cells and usually located at the upper part of the epididymis. The two cases seen were microscopically benign but locally invasive leading to obstruction of flow of sperm cells. Two cases were seen representing 1.2%. There was a case of chronic granulomatous inflammation induced by ova of schistosoma. The inflammation extended in to the epididymis causing distortion by fibrosis leading to obstruction.

Table 2 depicts the age distribution in relation to morphologic changes seen in the cases. The highest number of cases of male infertility occurred in the fourth decade of life with eighty three (83) reported cases followed by the 5th and third decades with 33 and 32 cases respectively.

Table 2: Age Distribution of the Various Testicular Lesions Causing Infertility

Morphology	Age Range			
	20 – 30	**31 – 40**	**41 – 50**	**> 50**
Obstruction/ Normal	7	24	13	5
Testicular Atrophy	7	26	5	7
Spermatocytic Arrest	7	10	9	3
Germ Cell Aplasia	9	14	4	-
Hypospermatogenesis	2	9	1	5
Adenomatoid Tumour	-	-	1	1
Total	32	83	33	21

DISCUSSION

Testicular lesions are varied and result from combined effects of various aetiological factors that include genetic, social and environmental. The differences in aetiologic and risk factors in various regions is probably the reason for the variation in the morphology of testes in the infertile males.

These lesions are major cause of both primary and secondary infertility and profiling them is important in determining the management and follow up of individuals with infertility.

Testicular biopsy and histology remain the gold standard in establishing the testicular cause of infertility (Levin, 1979). It is also useful in donor selection for therapeutic sperm retrieval and determination of individual's risk of developing a neoplasm. Lesions may differ from one testis to another in the same individual, therefore bilateral testicular biopsy is superior to unilateral in evaluation of patients with azoospermia and infertility (Plas *et al*., 1999).

The result of this study showed that, the most frequent morphologic features in the testes of the infertile male is normal morphology which constituted 29% of all the biopsies. This suggests a possible obstruction in the ductal system resulting from inflammatory and infective conditions, chronic hydrocele and tumours. Additional features in support of obstruction included sloughing and disorganization of the germ cells within the tubules. Even the single case of Schistosomiasis showed normal spermatogenesis but with marked fibrosis involving the duct system leading to obstruction and azoospermia. A relatively similar figure was reported by Wong *et al.* (1978) and Rashed *et al* (2008) but a study from Ibadan, Nigeria showed a much higher incidence of 38.2% (Thomas, 1990); Brannen and Roth (1979); and AlRayess *et al* (2000) also reported higher figures of 35% and 31% respectively. However, Meinhard *et al* (1973); Haddad *et al* (2004), Abdullah and Bondagji (2011) and Nagpal *et al* (1993) reported much lower figures representing 5%, 11.2%, 13% and 16% respectively. Obstruction in the duct system is amenable to treatment by microsurgical reconstruction leading to complete restoration of infertility.

The second most frequent morphologic alteration seen in the testes of the infertile males in this study was variable degrees of atrophy and hyalinization which accounted for 45 cases representing 28.4% of all the cases. This is a much higher incidence when compared with other studies. For example, a Nigerian study from the south-western part of the country by Thomas (1990) reported 22.4%. Jamal and Mansoor (2001) in Saudi Arabia reported 24% while much lower figures were reported by Nagpal *et al*. (1993) and Rashed *et al*. (2008). The reason for the high incidence of testicular atrophy and hyalinization is not clear but the aforementioned studies suggested previous inflammatory conditions of the testes, advancing age and undescended testes.

Spermatocytic arrest is the third most common histologic alteration seen in this study representing 17.2% of the total cases. Arrest may occur at any level of spermatogenesis but in this study, most occurred at primary to secondary spermatocytes and secondary spermatocytes to spermatids levels. Spermiogenic maturation arrest was not seen in this study. The causes of spermatocytic arrest are both genetic and acquired. The genetic causes include trisomies, deletions in the Y-chromosome etc. while the acquired include alcohol consumption, hypogonadism, chronic Indian hemp use etc. This finding is similar to that of Jamali and Haeri (1999), lower than that reported from Egypt by Rashed *et al* (2008) at 28% but much higher than those reported from Nigeria by Thomas (1990), Saudi Arabia by Abdullah and Bondagji (2011) and the Gulf by Haddad *et al* (2004) which represented 5%, 12% and 1.7% respectively.

Germ cell aplasia or Sertoli cell-only syndrome was reported in 27 patients representing 16% of all the lesions. This diagnosis was made when no germ cells are seen but Sertoli cells only in the seminiferous tubules. These findings were further confirmed by positive reactivity with immunohistochemical stain, vimentin. Sertoli cell-only syndrome is commonly associated with some structural chromosomal abnormalities (Vogt *et al*., 1996), cryptorchidism, and severe forms of orchitis, chemo- and radiation therapy.

This finding is similar to those from Saudi Arabia by Abdullah and Bondagji (2011) who also reported 16%, and 16.5% by Jamal and Mansoor (2001). Nagpal *et al* (1993) from India also reported a similar figure of 17%. In contrast, a study from Riyadh, Saudi Arabia by AlRayess (2000) reported a high figure of 39%.

Hypospermatogenesis is characterized by generalized decrease in numbers of germ cells in the seminiferous tubules. Typically, a patient with hypospermatogenesis should not present with azoospermia but oligospermia. In this study, patients were selected based on SFA result indicating azoospermia. This may be attributed to obstruction of the duct system or questionable SFA results which were conducted at different laboratories and were only brought in by the patients. Nevertheless, the morphologic findings were those of hypospermatogenesis. Hypospermatogenesis may be associated with congenital defects of germ cells, insensitivity to androgen and exposure to chemicals, heat and radiation. Cases of hypospermatogenesis were 17 representing 10.1%. This figure is much lower than those recorded by Mandong (2005) in Jos and Obafunwa *et al* (1993) in Lagos who recorded 52.5% and 49% respectively. Studies from Saudi Arabia by Abdullah and Bondagji (2011) reported 25% while that from India by Nagpal *et al* (1993) reported 42%. The reason for the lower incidence of hypospermatogenesis in this current study compared to those from other parts of Nigeria, Saudi Arabia and India is not very clear. Hypospermatogenesis was the most common histomorphologic alteration in the testes recorded by the studies from Jos, Lagos and India.

From the age distribution depicted in table 2, all the testicular lesions were most common in the third decade of life. Eighty three (83) out of the total of one sixty nine (169) cases of male infertility were in the age bracket of 31 to 40 years. This is probably the age bracket where exposure to the risk factors for infertility is maximal. In addition, marriages are done most commonly in that age bracket and a year or two after marriage without conception prompts couples to seek medical attention.

CONCLUSION

The study has highlighted the frequency and pattern of the various morphologic findings in the testes of infertile males with the most common being nsormal morphology signifying obstruction of the duct system. It also highlighted that, the highest incidence is in the 4th decade of life and further consolidated the relevance of Histopathological evaluation in the diagnosis and management of male infertility.

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