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ORIGINAL ARTICLE

ROLE OF FINE NEEDLE ASPIRATION CYTOLOGY IN THE EVALUATION OF MALE INFERTILITY

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ABSTRACT: BACKGROUND: Male infertility is a common problem and needs a minimally invasive method to arrive at the appropriate diagnosis. Alternative to open testicular biopsy the fine needle aspiration cytology of the testis is being increasingly used as a minimally invasive method of evaluating testicular function. **AIM OF THE STUDY:** To know whether FNAC of testes is as informative as biopsy in cases of male infertility. To establish that FNAC is cost effective, safe, outpatient investigation with no complications. **METHODOLOGY:** Fifty patients with primary male infertility in the age group of 20-40 years were included in the study. All the cases with oligospermia and azoospermia were subjected for Doppler study to rule out varicocele. Cord block was achieved with 1% lignocaine and aspiration was done with 23 gauge 1.5 inch needle. Smears were made on albuminised slides and stained with Leishman Stain. Forresta et al scoring system was adopted to analyse the smears. In the same sitting testicular biopsy was taken, fixed in Bouins fluid, routinely processed and stained with H&E stain. **RESULTS:** The commonest group with infertility were in the age group 21-30 years. On semen analysis 78% were azoospermic and 22% were oligozoospermic. The testicular size was normal in 90% of subjects and 10% had small testis. Out of fifty subjects with infertility, 40% subjects had varicocele. Varicocele was commonly associated with duct obstruction. The commonest patterns observed on cytology were; normal spermatogenesis (14/50, 28%), duct obstruction (8/50, 16%), maturation arrest (7/50, 14%) and testicular atrophy (7/50, 14%). In the present study diagnostic accuracy was 93.4%. **CONCLUSION:** Fine needle aspiration cytology is as informative as biopsy and can be done as a routine procedure. It is a simple and cost effective. In cases where FNAC shows normal spermatogenesis with azoospermia and oligospermia, biopsy and doppler study is indicated to rule out duct obstruction which can be corrected surgically.
KEYWORDS: Needle aspiration, male infertility, cytology, histology.

INTRODUCTION: Since times immemorial the wife has always been blamed for infertility, the possibility of treatment of the male for infertility is a recent one. Failure to find sperms in a post coital test, conducted by Max Hunner in 1913, raised the possibility that husband could be responsible for infertility. The available statistics show that the male factor is responsible for about 40% to 50% of all cases of sterility.

Assesment of male infertility involves clinical examination of gonads, secondary sexual characteristics, semen analysis, hormonal investigations and morphological examination of testicular biopsy. Open biopsy has proven to be important procedure to classify the pathogenesis of male infertility and to determine the prognosis. However this method is invasive and traumatic, requires anaesthesia which makes this technique difficult to use as a routine method.

Fine needle aspiration of testis is seldom performed in routine practice due to various reasons including poor compliance and apprehension on the part of the person performing the

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procedure. With many pathologists taking interest in FNAC there is standardisation of this procedure leading to a good correlation between cytology and histology of the testis. The present study is conducted to examine whether FNAC of testis is as effective as conventional biopsy so that unnecessary and expensive intervention can be avoided and to decide under what circumstances testicular biopsy is definitely indicated.

MATERIALS AND METHODS: Subjects with infertility who visited the Government General Hospital at a tertiary care center were included in the study. This is a prospective study for a period of two years. The age of the patients varied from 20-40years. After taking history and evaluating the patient clinically, semen analysis was done thrice with an interval of three weeks between the samples. Fifty subjects with primary infertility were analysed. All the fifty patients were subjected to Doppler study to diagnose varicocele. The subjects were subjected to FNAC and biopsy in the same sitting under aseptic conditions.

The procedure of aspiration was explained to the subject and after taking consent, the subject was placed in supine position, the testis is cleaned with spirit swab and draped in the usual sterile fashion. 2ml of 1% Lignocaine was infiltrated into the spermatic cord by fixing the vas deferens just beneath the scrotal skin using three finger technique. Lignocaine was injected along the vas deferns with a 25 gauge 1. 5 inch needle. A few minutes were allowed to lapse after the injection for the anesthetic effect. The testis was then grasped by an assistant who positioned the epididymis posterioly, so that anterior testis was directly beneath the stretched scrotal skin. The subcutaneous tissue along the lateral or medial aspect of the testis was then injected with 0. 5 to 1cc Lignocaine.

Care was taken to avoid injecting the anesthetic solution into tunica albuginea, so that underlying testicular architecture is not disturbed. Testicular sensation was elicited prior to and after the block was performed. The procedure of non-aspiration needle sampling was done in cases of infertility using a 23 gauge needle. Smears were immediately assessed for adequacy under the microscope and FNAC was repeated if necessary. The aspirate was immediately transferred to the albuminised slides and smear was made. The smears were air dried. In the same sitting with minimal scrotal incision open testicular biopsy was performed and fixed in Bouin's fluid. The incision was sutured with catgut and dressing made. They were no complications except for minimal pain and tenderness, which lasted for few hours. In cases surgical correction for varicocele was necessary the procedure was done in thereafter after spinal anesthesia.

FNAC smears were stained with Leishman's stain and was evaluated basing on the scoring system put forth by Foresta et al for male infertility. At least 500 cells were counted.

Scoring system based on Foresta et al.

Cytological Findings	Indices	Interpretation
Most of native cell seen	Sertoli index 50% or below	Normal
	Spermatic index 50% or below	
Most of native cells seen	Sertoli index above 50%	Hypospermatogenesis
	Spermatic index 50% or below	
Germ cells identified only to a particular level of maturation	Sertoli index 50% or below Spermatic index zero	Maturation arrest

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Germ cells identified only to a particular level of maturation	Sertoli index above 50% Spermatic index zero	Maturation arrest +Hypospermatogeneis
Spermatozoa out number other native cells	Sertoli index 50% or below Spermatic index above 50%	Duct obstruction
Sertoli cells only seen	Sertoli index 100% Spermatic index zero	Total atrophy/ Sertoli cell only syndrome

- Spermatic index (Number of spermatozoa per 100 spermatogenic cells.
- Sertoli Index (Number of sertoli cells per 100 cells counted).

The smears were grouped under six headings:

1. Normal.
2. Hypospermatogenesis.
3. Maturation arrest.
4. Maturation arrest with hypospermatogenesis.
5. Duct obstruction.
6. Total atrophy/Sertoli cell only syndrome.

The testicular biopsies were stained with Hematoxylin and Eosin after routine processing. Biopsy sections grouped into five categories:

1. Normal spermatogenesis.
2. Hypospermatogenesis.
3. Maturation arrest.
4. Germ cell aplsia, Sertoli cell only syndrome.
5. Tubular hyalinisation or tubular sclerosis.

RESULTS: In the present study fine needle aspiration was done on 50 patients who had azoospermia or oligospermia on semen analysis. The commonest group with infertility were in the age group 21-30years (n=32, 64%). (Table 1) On semen analysis 39/50 (78%) were azoospermic and 11/50(22%) were oligozoospermic with grade IV motility >50% in 27.2% (3/11) and <50% in 72.7% (8/11) of subjects.

The testicular size was normal in 90 %(45/50) of subjects and 10 %(5/50) had small testis. In one case there was female distribution of fat, gynecomastia and small size of testis. This was clinically suspected to be Klinefelters syndrome and confirmed on histopathology and karyotyping. Out of five cases with small sized testis four cases had testicular atrophy secondary to mumps infection on histopathology and four cases with normal sized testis had testicular atrophy on histopathology.

Out of fifty subjects with infertility, 20(40%) subjects had varicocele. Twenty five percent cases were clinically diagnosed as having varicocele and rest of 75% (15cases) was identified on Doppler study. Varicocele is commonly associated with duct obstruction. Out of 10 cases with features of duct obstruction on histopathology, 60 %(6 cases) had bilateral varicocele and 4 cases (40%) had unilateral varicocele predominantly on left side. Three cases had normal

spermatogenesis, two cases had hypospermatogenesis, two cases had maturation arrest, two cases had testicular atrophy and sertoli cell syndrome on histopathology, in one case the biopsy was inadequate. (Table 2).

The commonest patterns observed on cytology were; normal spermatogenesis (14/50, 28%), duct obstruction (8/50, 16%), maturation arrest (7/50, 14%) and testicular atrophy (7/50, 14%). In two cases needle aspiration did not yield any cellularity, they are diagnosed as testicular atrophy on histopathology. (Table 3) The commonest patterns observed on histopathology were: duct obstruction (12/50, 24%), testicular atrophy (9/50, 18%), normal spermatogenesis (7/50, 14%) and maturation arrest (7/50, 14%).

Seven cases with diagnosis of normal spermatogenesis on biopsy 5 cases presented with azoospermia and 2 with oligospermia. Four cases with diagnosis of hypospermatogenesis on biopsy 2 cases presented with azoospermia. This could be due to presence of sperm antibodies, which was not possible in the present study. Out of 12 cases with duct obstruction on histopathology, 8 cases presented with azoospermia and four with oligospermia, this could be due to partial or complete obstruction.

Out of 50 cases studied 2 cases are inadequate for histopathological study, but on cytology they showed normal spermatogenesis. In two cases FNA yielded only blood which were diagnosed as testicular atrophy on histopathology. In both the cases the tunica albuginea was very much thickened, hence did not yield any cellularity on FNAC. Out of 46 cases available in 43 cases (93. 4%) showed correlation between cytology and histopathology. In 3 cases no correlation was seen between cytology and histopathology. These 3 cases were diagnosed as duct obstruction on histopathology and normal spermatogenesis on cytology. This error could be due to the broader range given in the scoring system for duct obstruction on FNAC. (Table 4).

In the present study histopathology showed normal spermatogenesis with focal atrophy in 2 cases. Their semen analysis showed oligozoospermia with FNAC showing normal spermatogenesis. In 3 cases tubules with hypercurvature are noticed, FNAC showing normal spermatogenesis. In cases of duct obstruction, normal spermatogenesis with focal atrophy and tubules with hypercurvature cytology did not give any information suggestive of these conditions. Hence testicular biopsy is very much indicated in the above three conditions. In cases of testicular atrophy, sertoli cell only syndrome, hypospermatogenesis, maturation arrest, cytology is as informative as histopathology.

DISCUSSION: The assessment of male infertility involves a clinical examination for gonadal development and secondary sexual characteristics, semen analysis, hormonal investigations and morphological examination of testicular biopsy. Open biopsy has proven to be an important procedure to clarify the pathogenesis of male infertility and to determine the prognosis. However this method is invasive and traumatic and the risk increases when done on both sides. Therefore needle puncture has been proposed as an alternative method.

Posner and Huhner first used testicular puncture biopsies in the investigation of human infertility that examined unstained samples for spermatozoa.[1] Later fine needle aspiration of the testis pioneered by Obrant and Persson (1965) was proposed as a non-invasive technique.[2] Characterizing the cell types in cytological smears was straightforward, with not much difficulty in recognizing germ cells and Sertoli cells. The material aspirated by FNAC is adequate and the various cell types can be identified by their distinctive morphology. Some authors have attempted to

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quantitatively analyze the population of germ cells. Proportion of Sertoli cells, other germ cells and spermatozoa help in arriving at the diagnosis on cytological smears.[3]

In the study by Abraham Kurien et al.[4] a total of 57 patients were analysed. In their study three men out of the total had unilateral testis. The mean age of the patients studied was 29. 9 years with a range from 22 years to 56 years. The duration of infertility of the patients studied ranged from 1 year to 15 years with a mean duration of 4. 9 years. Almost all patients were primarily infertile except for 3 patients who were having secondary infertility. Twelve patients were detected to have varicoceles, 37 patients were confirmed to be azoospermic after 3 semen analyses and the remaining were oligospermic.

In the study by S. P. Yadav et al.[5] analysed 25 patients with infertility, the largest group belonged to the age group of 26-30 years, seeking advice after 4-6 years of mutual cohabitation. Twenty two cases presented with primary infertility and 3 with secondary infertility. 4% presented with left sided varicocele, 21 patients were azoospermic and 4 patients with oligospermia. 69% had normal sized testis with histopathological diagnosis of normal spermatogenesis, 62.5% had small sized testis with diagnosis of sertoli cell only syndrome or testicular atrophy.

In the present study 50 cases of male infertility were subjected to fine needle aspiration. Maximum number of patients presented with infertility between 21-30 years. They consulted the physician after 2-4 years of married life. All these cases presented with primary infertility. Thirty nine cases (78%) were azoospermic with 18% of cases associated with bilateral varicocele, and 11 cases (22%) were oligozoospermic. The testicular size was normal in 90% of cases and in 10% of cases the size was small for age. Five cases with normal sized testis showed features of testicular atrophy on histopathology. Unilateral varicocele was seen in 4 cases mainly on the left side.

The various cytological patterns in the present study compared with other studies were:

Cytological patterns	**Rajwanshi et al [6]**	**Gottschalk et al [7]**	**Qublan HS et al [8]**	**U. R. Parikh et al[9]**	**Dr. Abraham Kurien et al [4]**	**Present study**
Normal spermatogenesiss	49%	37%	20. 6%	35. 09%	50. 5%	28%
Hypospermatogenesis	-	-	26. 5%	-	15. 3%	8%
Maturation arrest	30%	18. 5%	23. 5%	11. 25%	11. 7%	14%
Sertoli cell only syndrome	10%	31. 5%	29. 4%	18. 75%	3. 6%	4%
Testicular atrophy	5%	-	-	11. 25	12. 6%	14%
Insufficient material	6%	13%	4. 4%	-	-	4%

The accuracy of fine needle aspiration cytology was determined by comparing the FNAC findings with that of the histological findings obtained from an open surgical biopsy, which was taken as the gold standard for the diagnosis. In the study by Abraham Kurien et al there was good agreement between the cytological and histological diagnosis. An accuracy of 91.9% was achieved in their study. In diagnosing a correctable post testicular cause for infertility in those patients with

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azoospermia and cytology proven normal spermatogenesis the accuracy of fine needle aspiration cytology compared to histopathology was 100%. Madhu S Agarwal et al.[10], Forresta C. Varatto et al.[3], Eric Piaton et al.[11], Han U et al.[12] showed accuracy of 100%, 92%, 72.2%, 91.6% respectively. In the present study the correlation percentage was 93. 4%.

CONCLUSIONS:

1. The technique of testicular FNAC is simple, inexpensive and minimally traumatic. More than 1 specimen can be taken safely.
2. Testicular FNAC gives an accuracy of 93. 4% in the diagnosis of patients with male infertility.
3. The material aspirated by FNAC is adequate and the various cell types can be identified by their distinctive morphology. This study proves that FNAC can evaluate accurately all classically defined histologic types.
4. FNAC obtained insufficient smears mainly in atrophied testes.
5. The accuracy of diagnosing normal spermatogenic activity in obstructive azoospermia by FNAC was 100%.
6. For evaluating the spermatogenic activity in male infertility it appears that a unilateral FNAC or biopsy is sufficient for diagnosis. Bilateral FNACs and biopsies can be restricted to patients in whom there is appreciable difference in testicular size or consistency.
7. In cases of duct obstruction, normal spermatogenesis with focal atrophy and tubules with hyper curvature cytology did not give any information suggestive of these conditions. Hence testicular biopsy is very much indicated in the above conditions.
8. In cases of testicular atrophy, sertoli cell only syndrome, hypo spermatogenesis, maturation arrest, cytology is as informative as histopathology.

Age	No. of cases (n=50)	Percentage
10-20 years	1	2
21-30 years	32	64
31-40 years	17	34

Table 1: Age wise distribution of male infertility cases

Morphological Types on biopsy	No. of cases (n=50)	Physical Evaluation	Testicular size	Bilateral varicocele (Doppler)	Unilateral varicocele (Left side)	Unilateral varicocele (Right side)
Normal Spermatogenesis	7	Normal	Normal	1	2	-
Hypospermato genesis	4	Normal	Normal	1	1	-
Hypospermato genesis with maturation arrest	4	Normal	Normal	-	-	-
Maturation arrest	7	Normal	Normal	1	1	-

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Duct obstruction	12	Normal	Normal	6	3	1
Sertoli cell only syndrome	2	Normal	Normal	-	-	1
Klinefelters syndrome	2	Gyneco mastia	Small	-	-	-
Interstitial orchitis	1	Normal	Normal	-	-	-
Testicular atrophy	9	Normal	5-normal 4-small	1	-	-
Inadequate biopsy	2	Normal	Normal	1	-	-

Table 2: Clinical, Morphological and Radiological evaluation of cases of Male infertility

Morphological diagnosis on Cytology	Number of cases (n=50)	Percentage
Normal Spermatogenesis	14	28
Hypospermato genesis	4	8
Hypospermato genesis with maturation arrest	3	6
Maturation arrest	7	14
Duct obstruction	8	16
Sertoli cell only syndrome	2	4
Klinefelters syndrome	2	4
Interstitial orchitis	1	2
Testicular atrophy	7	14
Inadequate material	2	4

Table 3: Patterns on cytology

Cases	Diagnosis on cytology	Correlated cases	Non correlated cases
Normal Spermatogenesis	12	9	3
Hypospermato genesis	4	4	-
Hypospermato genesis with maturation arrest	3	3	-
Maturation arrest	7	7	-
Duct obstruction	8	8	-
Sertoli cell only syndrome	2	2	-
Klinefelters syndrome	2	2	-
Interstitial orchitis	1	1	-
Testicular atrophy	7	7	-

Table 4: Cyto-Histopathological correlation; 46 cases available

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Fig. 1: FNA smear of Maturation arrest showing germ cells identified only to particular level of maturation (Leishman stain, 400x)

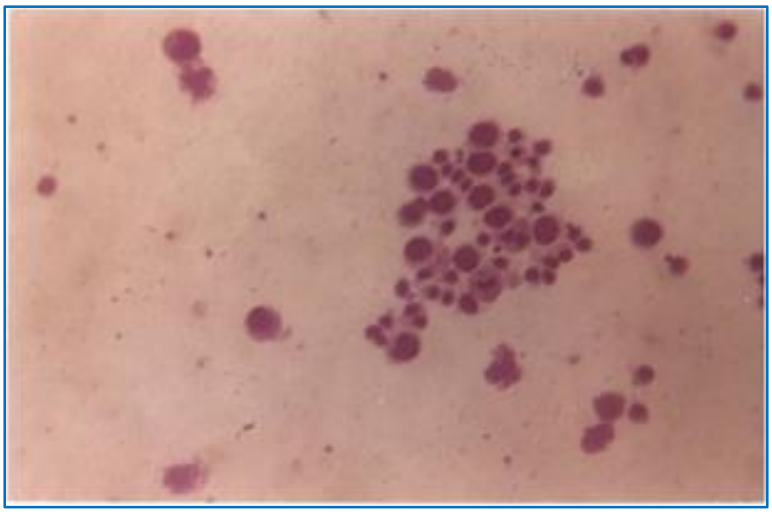

Fig. 1

Fig. 1A: Histopathology of maturation arrest (H&E, 200x)

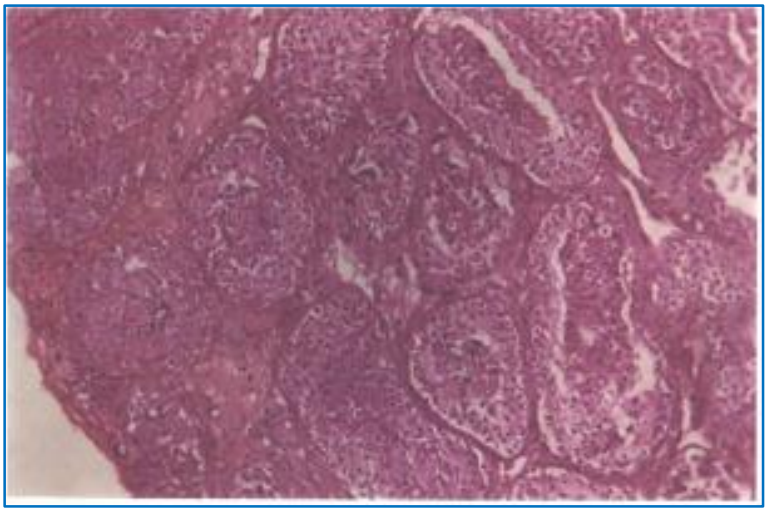

Fig. 1(A)

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Fig. 2: FNA smear of Duct obstruction showing spermatozoa out number other native cells (Leishman stain, 400x).

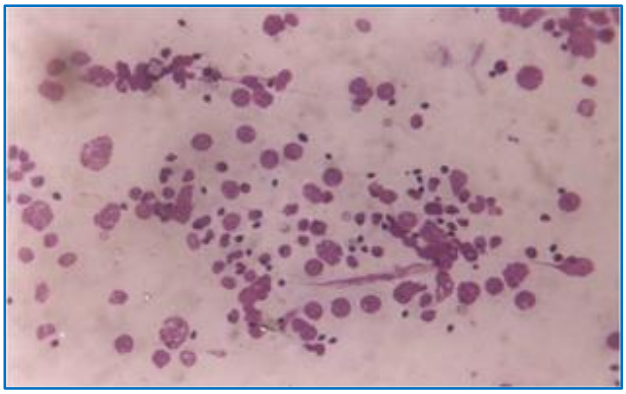

Fig. 2

Fig 2 A: Histopathology of Duct obstruction (H&E, 400x).

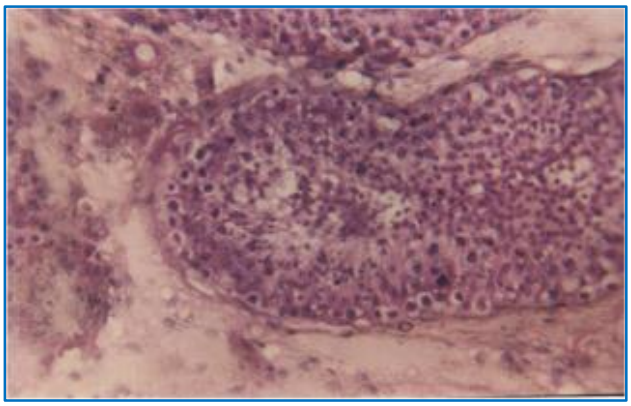

Fig. 2(A)

Fig. 3: FNA smear of Sertoli cell only syndrome showing Sertoli cells only (Leishman stain, 200x).

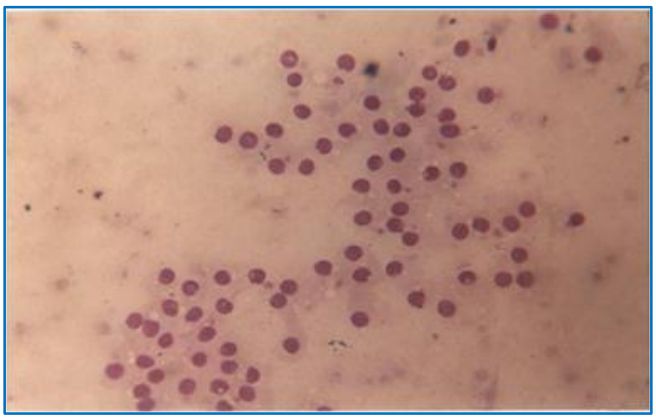

Fig. 3

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Fig. 3A: Histopathology of Sertoli cell only syndrome (H&E, 400x).

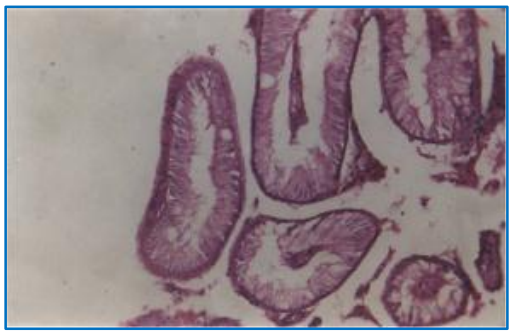

Fig. 3(A)

Fig. 4: FNA smear of Testicular atrophy showing Sertoli cells only (Leishman stain, 200x)

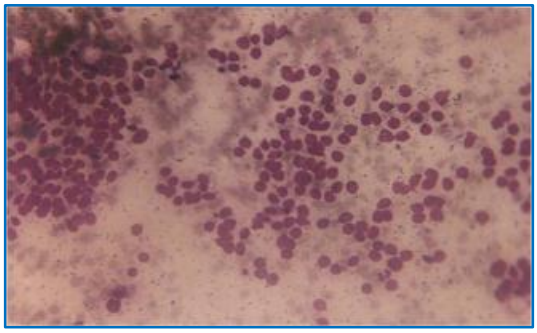

Fig. 4

Fig 4A: Histopathology of Testicular atrophy (H&E, 400x)

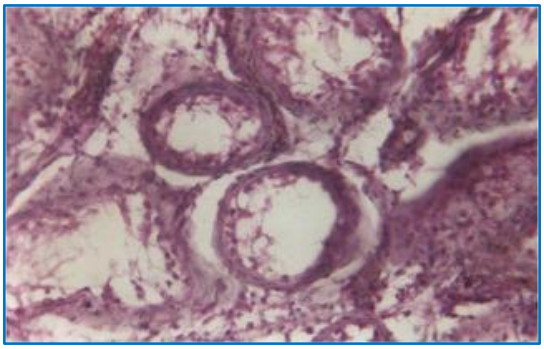

Fig. 4 (A)

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