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Morphological Changes in the Testis of White Rats with Hyerprolactinaemia

R. Hasan
Senior Lecturer, Department of Anatomy, Faculty of Medical Sciences, University of Kelaniya

Abstract**—***Male factor infertility due to endocrine disturbances such as abnormalities in prolactin levels are encountered in a significant proportion of infertile patients. [1]. This case control study was carried out to determine the effects of increased prolactin levels on the morphology of the testis, using 200 male white rats belonging to the wistar strain. The rats were maintained as the control group (G1), 3 hyperprolactinaemic groups induced by using oral largactil (G2), low dose fluphenazine (G3) and high dose fluphenazine (G4). After 100 days, the rats were subjected to serum prolactin (PRL) level measurements. The difference between serum PRL concentrations of rats in G2, G3, and G4 as compared to the control group were highly significant by Student's t-test ($p<0.001$). Thus it is evident that spermatogenesis is arrested at or before the elongated spermatid formation whereby there is a marked reduction in cellularity and spermatozoa. It is also evident that a few Sertoli cells with a few elongated spermatids attached to them do occur.*

Keywords**—** *Male factor infertility, Hyperprolactinemia, Morphological changes, testis, rats.*

I. INTRODUCTION

Infertility is defined as the failure of a couple to conceive after at least 12 months of unprotected intercourse [1]. Infertility in a couple can be described as male factors, female factors or issues in both partners, while the male reproductive capacity was found to be deficient in more than 50% of infertile couples [1]. A link between male factor infertility and low sperm count in hyperprolactinaemic patients has been described but the exact mechanism is unknown [2].

Pre-testicular causes account for up to 10% of male factor infertility and mainly include hormonal factors namely, follicular stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL). Elevated levels of PRL have been shown to result in drastic inhibition of sperm production and its quality [2]. Prolactin abnormality can result from trauma, tumours in the pituitary gland, malfunction of the pituitary gland, chronic liver disease, thyroid dysfunction and genetic and chromosomal defects such as the Klinefelter syndrome [3]. Hyperprolactinaemia has been known to cause male factor infertility resulting in decreased libido and impotence. Treatment with bromocriptine to suppress the elevated PRL level has been very successful in reversing the condition and achieving a pregnancy. The role of PRL on the morphology of the testis has been shown in only a few studies and the exact role in male factor infertility remains unclear. Hence this study was carried out to fill this void to a certain extent.

II. METHODOLOGY

The study was a case control type and was carried out in the Animal House of the Faculty of Medicine, University of Ruhuna, Sri Lanka. The objective of this study was to determine the effects of prolactin on the morphology of the male reproductive tract in otherwise normal rats and thereby to determine whether abnormality of PRL levels is a contributory factor to infertility in males. Ethical consent for this study was obtained from Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka. Male white rats of the Wistar strain were obtained from the Medical Research Institute, Borella, Colombo and also from breeding carried out at the Animal House, Faculty of Medicine, University of Ruhuna. 10 ± 2 week old rats weighing 200 ± 10 g were maintained at a room temperature of 28 ± 4 degrees Celsius and fed with animal feed for a period of 2 weeks. The quantity of feed and volume of water consumed by the rats was measured and recorded on a daily basis. 200 rats were selected and grouped from GI to G4. 30 rats were included in each group and maintained in separately labeled cages.

These groups were subjected to the following procedures. Group 1 (G1) — The 30 rats in this group were maintained under normal conditions at room temperature, in order to obtain a control value for the normal serum PRL level of rats. Group 2 (G2) — The 30 rats in this group were fed with oral largactil in a dose of 10mg per kg body weight per day in a divided dose given twice a day, dissolved in 2m1 of distilled water. Another lot of 30 male white rats, age and weight matched, were fed with an equal volume of distilled water and served as a control. A daily chart of food intake, drugs intake, and fluid intake and body weights was maintained. Group 3(G3) — The 30 rats in this group were treated with daily subcutaneous injections of fluphenazine in sesame oil in a dose of 0.42 mg per kg body weight per day in a single dose given in the morning. A daily chart of food intake, drugs intake, and fluid intake and body weights was maintained. Group 4 (G4) — The 30 rats in this group were treated identically as with group 3 except that the dose of fluphenazine was increased to 0.84 mg per kg body weight per day. Group 5 (G5) — The 30 rats in this group served as the control for the rats treated with subcutaneous injections of fluphenazine in groups 3 and 4. They were given an equal volume of sesame oil as injections. Hyperprolactinaemia was induced in the rats using oral largactil and subcutaneous injections of fluphenazine. The dosages of drugs used in the induction of experimental variations in serum PRL concentrations were obtained from the British National Formulary. The oral drugs were dissolved in measured volumes of distilled water and administered to the rats using a feeding tube. The feeding was done over a period of 100 days.

A. Assessment of Prolactin (PRL) levels in Rat Serum

At the end of 100 days 20 rats from each group were subjected to serum PRL assays by drawing 2ml of blood using sterile plastic disposable syringes under aseptic conditions. The PRL concentrations of rats were measured using the immulite random access chemiluminescent immunoassay method machine. The machine used in the study has a sensitivity of 0.5ng/m1 for PRL measurements. Many samples of rat serum would have PRL concentrations below this amount and would therefore not be read by the machine. In order to overcome this difficulty the procedure adapted was modified as follows. 100µl of rat serum was mixed with an equal volume of serum obtained from a male human volunteer with previously estimated PRL concentration. The blood samples from the donor were obtained and the 4 samples mixed. The mean value for serum PRL concentration of the donor sample obtained from 4 assays done on different days was 7.3ng/ml. Following the assays the concentration of PRL in the rat serum was calculated by difference from the value for the human serum alone. A strict parallelism test involving recovery of added known quantities of rat serum PRL was not possible in this study due to the unavailability of the necessary rat hormone in pure form. To compensate for this, studies were carried out utilizing different volumes of rat serum (spiking recovery test).

B. Obtaining and the Preparation of Tissues of Testis of Rats for Light Microscopic Studies

Three rats from each of the above groups were subjected to light microscopic studies. Each rat was anaesthetized using ether in a dessicator and dissected. The cardiac chambers were identified, and 5 ml of 10 % formalin was injected into the left ventricle. The male reproductive tract of the rat was dissected and preserved in 10% formalin for 5 days. Sections of the testis from the upper, middle and lower poles were obtained using a new razor blade, washed over night with tap water, labeled and tied up individually in small sacs of surgical gauze. Thereafter they were stained with haematoxylin and eosin for light microscopic studies.

III. RESULTS

The results of the morphological studies under light microscopy are illustrated in figures 1 to 13, with figures 1, 2 & 3 illustrating the normal tissue morphology of the testis as was evident in tissues obtained from the control group.

In the control group, the cellular arrangement is typically confined to the area adjacent to the basement membrane with a marked concentration of spermatogonia close to the basement membrane. Numerous spermatocytes are also seen.

Figures 4 to 7 illustrate the testicular tissue morphology of rats treated with oral largactil. The cellular arrangement within the seminiferous tubule is disturbed with spermatogonia sighted close to the basement membrane. A few spermatids (round and elongated forms) are visible. Both round and elongated spermatids are attached to Sertoli cells; the elongated spermatids tend to extend into the lumen.

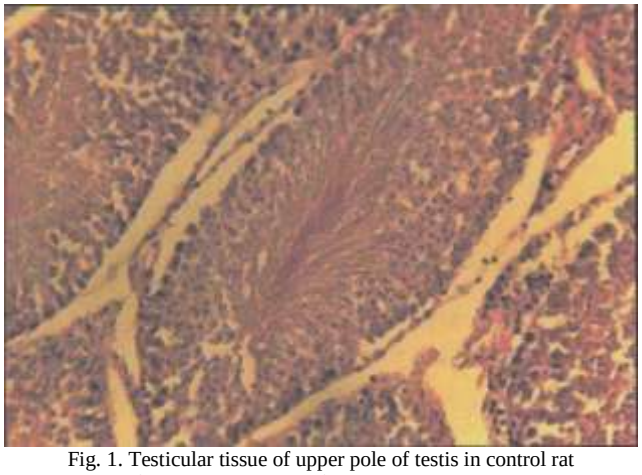

Fig. 1. Testicular tissue of upper pole of testis in control rat
Section prepared from the upper pole of the testis (H&E /LM X 66)

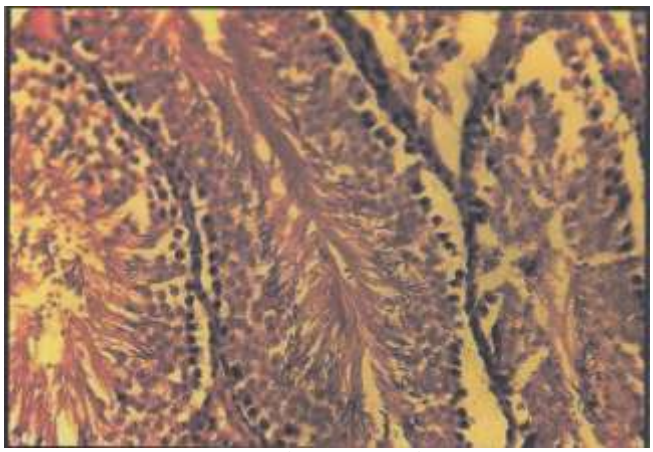

Fig. 2. Testicular tissue of middle pole of testis in control rat
Section prepared from the middle pole of the testis. (H&E/LM X 66)
The cellular arrangement is typically confined to the area adjacent to the basement membrane. Marked concentrations of spermatogonia are seen close to the basement membrane, a few with active nuclei (type B spermatogonia) and others with small nuclei (type A spermatogonia). Numerous spermatocytes are seen.
Both round and elongated spermatids are seen, attached to Sertoli cells; the elongated spermatids tend to extend into the lumen.
Mature spermatozoa are seen in large numbers within the lumen

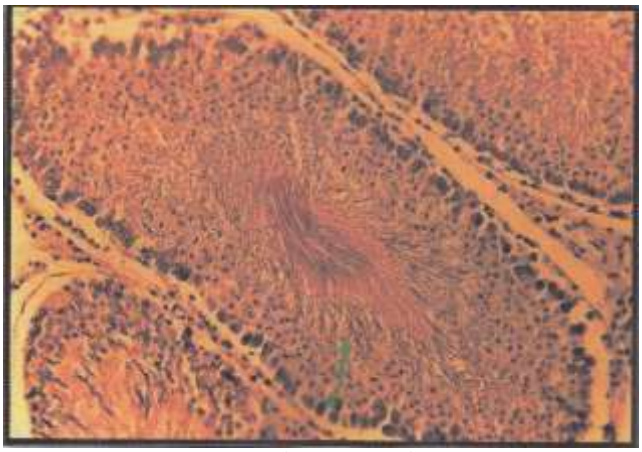

Fig. 3. Testicular tissue of lower pole of testis in control rat
Section prepared from the lower pole of the testis (H&E/ LM X 132)
The normal cellular distribution around the periphery of the seminiferous tubules, is seen with numerous spermatogonia and spermatocytes. The lumen is filled with spermatozoa (a). Elongated and round spermatids are seen in the tubule.

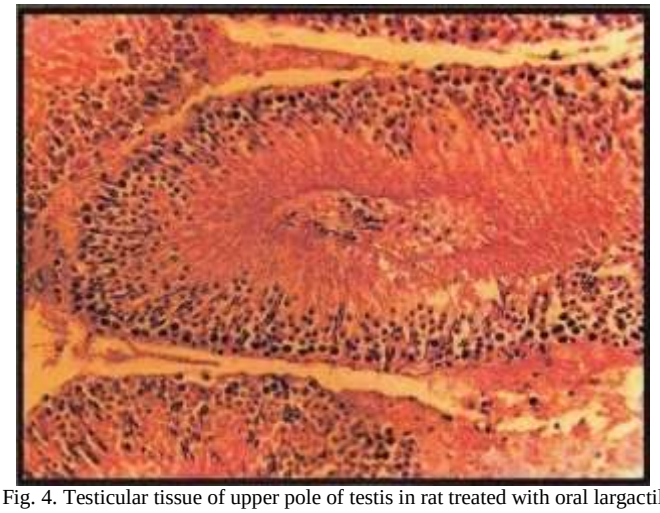

Fig. 4. Testicular tissue of upper pole of testis in rat treated with oral largactil
Section prepared from the upper pole of the testis (H&E/LM x 66)
The cellular arrangement within the seminiferous tubule is disturbed. Spermatogonia are seen close to the basement membrane. A few spermatids (round and elongated forms) can be identified.

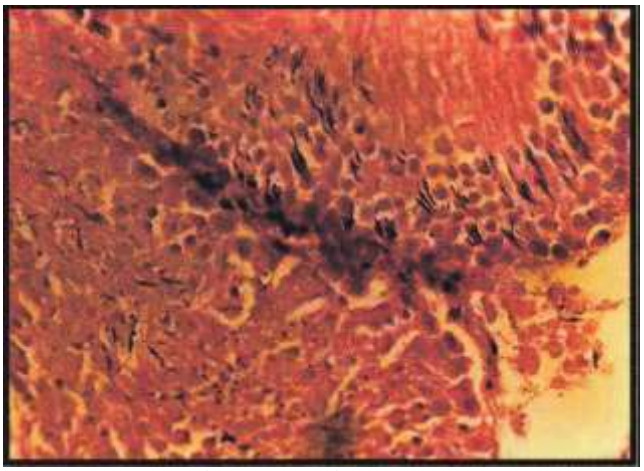

Fig. 5. Testicular tissue of middle pole of testis in rat treated with oral largactil
Section prepared from the middle pole of the testis (H&E/LM x 132)
Note the reduction in cellularity and the presence of elongated spermatids which are freely extending in to the lumen. A few round spermatids can also be identified.

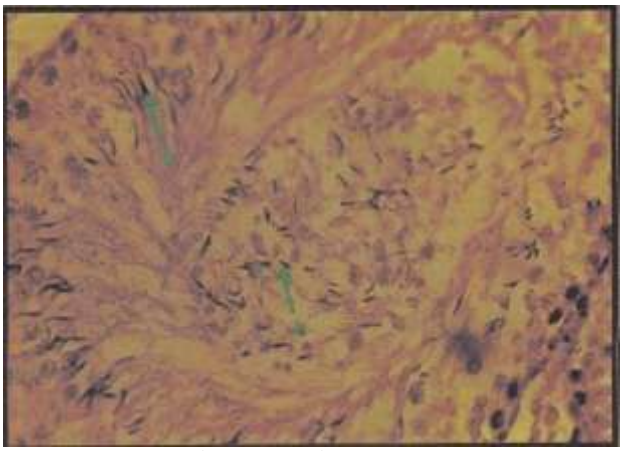

Fig. 6. Testicular tissue of lower pole of testis in rat treated with oral largactil
Section prepared from the lower pole of the testis (H&E/LM x 132)
Numerous elongated spermatids (a) and some cellular debris are seen within the lumen. A few sperm heads (b) are also seen towards the periphery of the tubular lumen.

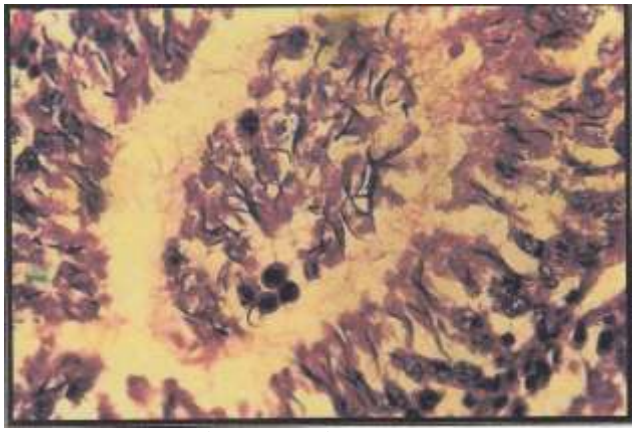

Fig. 7. Testicular tissue of lower pole of testis in rat treated with oral largactil
Section prepared from the lower pole of the testis (H&E/LM x 330)
Numerous elongated spermatids are seen within the lumen. A few round spermatids are also seen. Spermatozoa are hardly recognizable in this section.

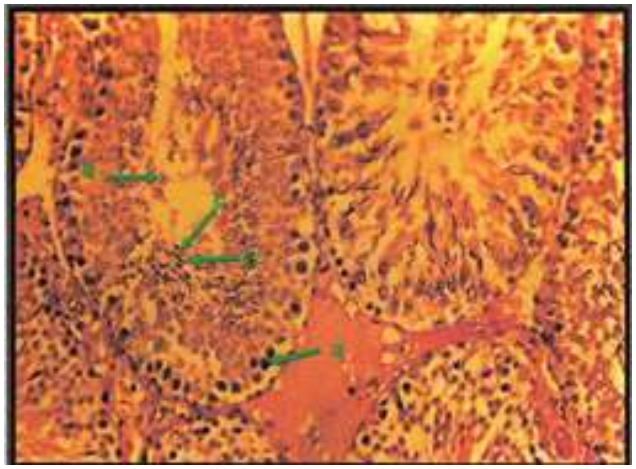

Fig. 8. Testicular tissue of upper pole of testis in rat treated with low dose of fluphenazine
Section prepared from the upper pole of the testis (H&E/LM x 66)
Spermatogenesis is arrested at or before elongated spermatid formation.
There is a marked reduction in the cellularity. Spermatogonia (a) are seen adjacent to the basement membrane. A few elongated spermatids (b), round spermatids (c) and cellular debris (d) is seen within the lumen. Note the marked reduction in spermatozoa.

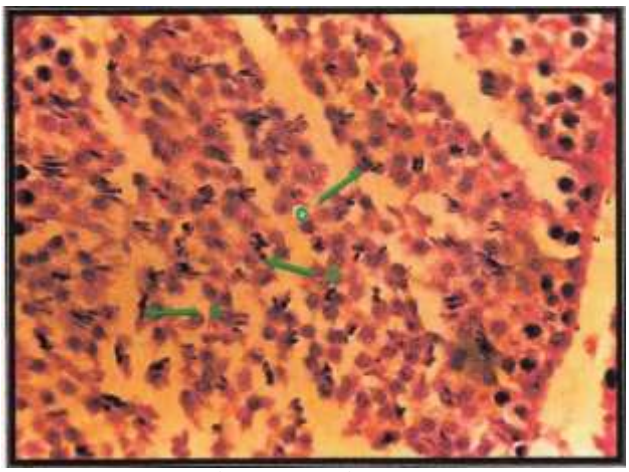

Fig. 9a. Testicular tissue of middle pole of testis in rat treated with low dose of fluphenazine
Section prepared from the middle pole of the testis (H&E/LM x 132)

Numerous elongated spermatids (a), a few round spermatids (b) and cellular debris (c) is seen within the lumen. Spermatozoa cannot be seen in this section.

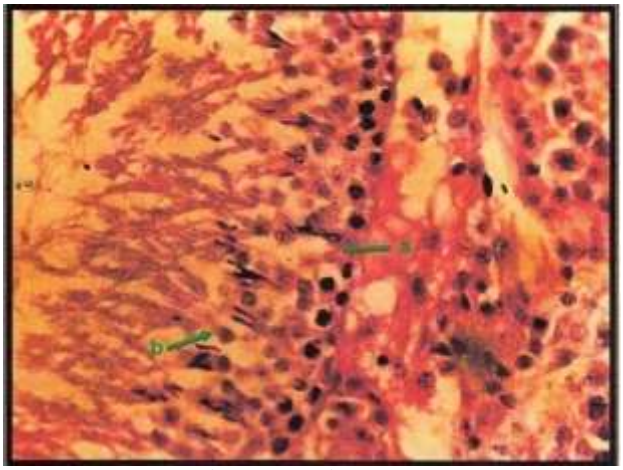

Fig. 9b. Testicular tissue of lower pole of testis in rat treated with low dose of fluphenazine
Section prepared from the lower pole of the testis (H&E/LM x 132)
Section shows a Sertoli (a) cell with a few elongated spermatids attached to it. The rest of the Sertoli cells tend to be free of spermatids. A few secondary spermatocytes (b) are also seen.

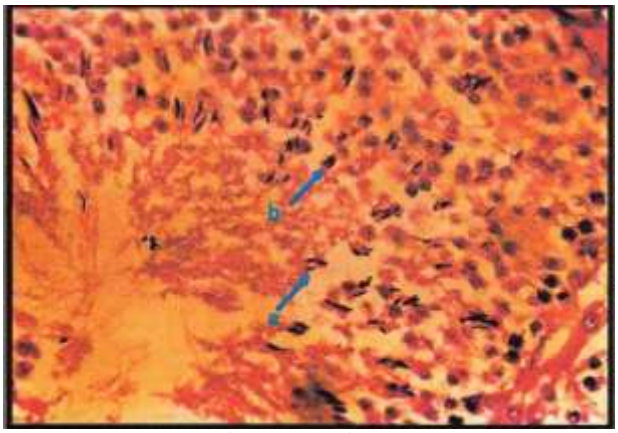

Fig. 9c. Testicular tissue of lower pole of testis in rat treated low dose of fluphenazine
Section prepared from the lower pole of the testis (H&E/LM x 132)
Secondary spermatocytes, numerous elongated spermatids (a) and few round spermatids (b) are seen toward the lumen.

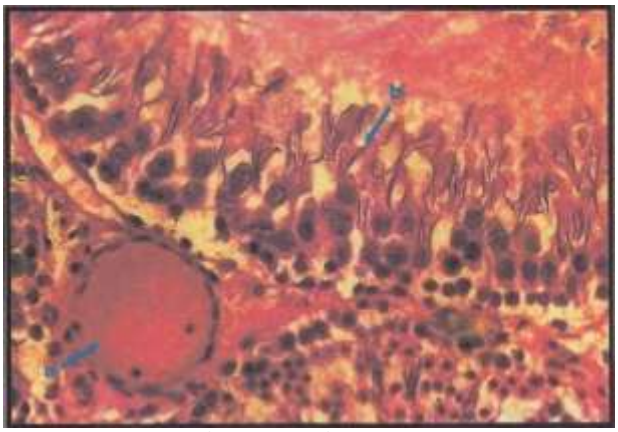

Fig. 9d. Testicular tissue of lower pole of testis in rat treated with low dose of fluphenazine
Section prepared from the lower pole of the testis (H&E/LM x 132)
Spermatogenesis is arrested at elongated spermatid formation.
A blood vessel (a) cut in section in interstitium. Spermatogonia, secondary spermatocytes and Sertoli cells with spermatids attached are seen.
Cellular debris within the lumen. Numerous elongated spermatids (b) extend into the tubular lumen. Spermatozoa are not visible in this section.

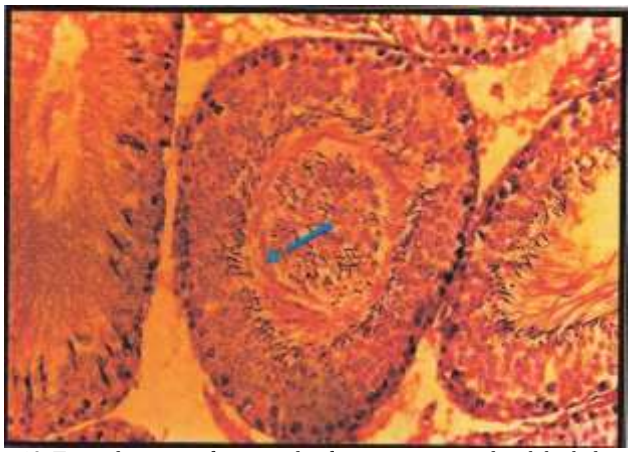

Fig. 10. Testicular tissue of upper pole of testis in rat treated with high dose of fluphenazine
Section prepared from the upper pole of the testis (H&E/LM x 66)
Spermatogenesis is arrested at or before round spermatid formation. Spermatocytes and round spermatids are seen. Numerous cells (a) are seen within the lumen. It is possible that these cells have lost their physiological function and are thus shed into the lumen. Elongated spermatids are hardly seen in this section.

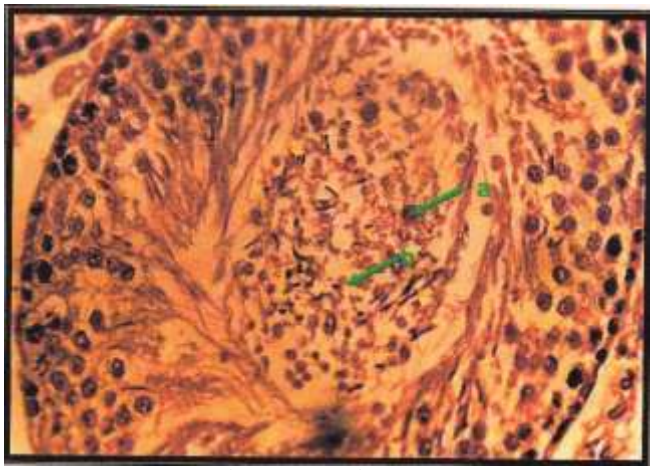

Fig. 11. Testicular tissue of middle pole of testis in rat treated with high dose of fluphenazine
Section prepared from the middle pole of the testis (H&E/LM x 132)
Spermatogenesis is arrested at or before round spermatid formation. A reduction in cellularity with a few round spermatids (b) are seen. Elongated spermatids are not visible in this section. Cellular debris (a) is seen within the lumen.

Figures 8 to 9 illustrate the testicular tissue morphology of rats treated with low dose of fluphenazine. It is clearly evident that spermatogenesis is arrested at or before the formation of elongated spermatids. There is a marked reduction in the cellularity. Spermatogonia are seen adjacent to the basement membrane. A few elongated spermatids, round spermatids and cellular debris is seen within the lumen. There is marked reduction in spermatozoa.

Figures 10 to 13 illustrate the testicular tissue morphology of rats treated with high dose of fluphenazine. It is evident that spermatogenesis is arrested at or before the formation of round

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spermatids. Spermatocytes and round spermatids are seen. Numerous cells are seen within the lumen. It is possible that these cells have lost their physiological function and are thus shed into the lumen. Elongated spermatids are hardly seen in this section. The cellular arrangement within the seminiferous tubule is disturbed with a reduction in cellularity and the presence of spermatogonia close to the basement membrane. A few spermatids are seen freely extending into the lumen. Also a few sperm heads are visible towards the periphery of the tubular lumen which may have some cellular debris. Spermatozoa are hardly recognizable.

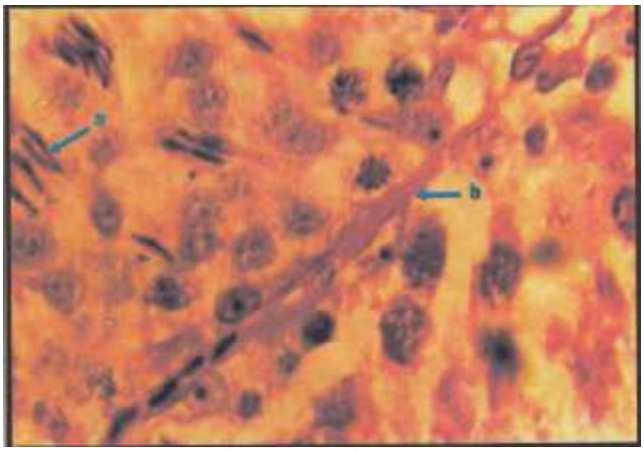

Fig. 12. Testicular tissue of middle pole of testis in rat treated with high dose of fluphenazine
Section prepared from the middle pole of the testis (H&E/LM x 330)
Elongated spermatids (a) are found freely within the lumen. Spermatogonia and primary spermatocytes are seen adjacent to the basement membrane (b).

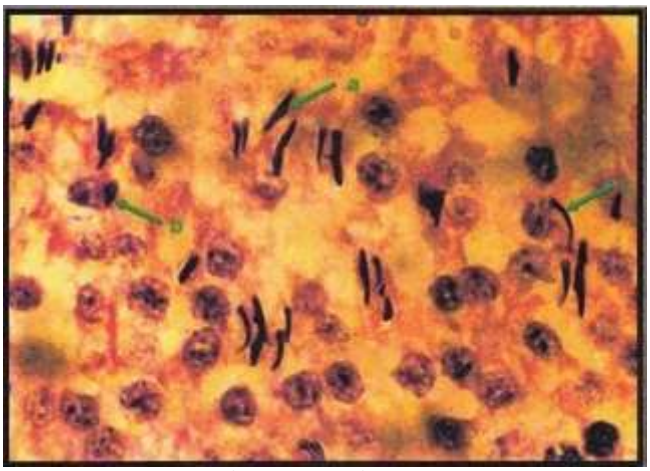

Fig. 13a. Testicular tissue of middle pole of testis in rat treated with high dose of fluphenazine
Section prepared from the middle pole of the testis (H&E/LM x 330)
Elongated spermatids (a) together with a few round spermatids (b) are seen lying freely. A few spermatozoa (c) and numerous secondary spermatocytes are visible.

Thus it is evident that spermatogenesis is arrested at or before the elongated spermatid formation whereby there is a marked reduction in cellularity and spermatozoa. It is also evident that a few Sertoli cells with a few elongated spermatids attached to them do occur.

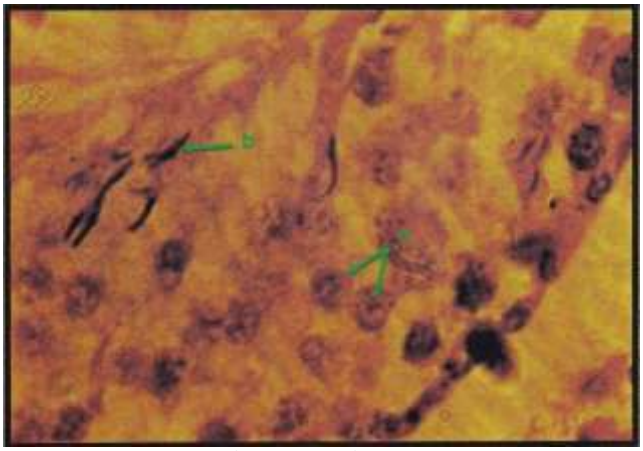

Fig. 13b. Testicular tissue of lower pole of testis in rat treated with high dose of fluphenazine
Section prepared from the lower pole of the testis (H&E/LM x 330)
A high populations of secondary spermatocytes (a) and a few elongated spermatids (b) are seen.

IV. Discussion

From the results of the morphological studies carried out under light microscopy it appears that increasing PRL concentrations have a strong bearing on the process of spermatogenesis. These findings are augmented to an extent by the findings of Negro-Vilar et al. [4] who have reported the existence of a relationship between alterations in endogenous PRL levels and testicular function in developing rats. However, the latter have not reported conclusive findings with regard to the effect of abnormal PRL levels on the testis in rats.

In the case of hyperprolactinaemia, according to the results of the testicular studies carried out it appears that spermatogenesis is affected at the spermatid stage formation, possibly due to damage to Sertoli cells. Under normal circumstances spermatids complete their maturation into elongated spermatids while being attached to the Sertoli cells. However damage to Sertoli cells as in the case of this research experiment impairs this maturation process whereby incompletely mature spermatids are released into the lumen of the seminiferous tubule. Under normal circumstances spermatids complete their maturation while being attached to Sertoli cells and are not found within the lumen of the seminiferous tubules. It is possible that the spermatid binding to the Sertoli cell is impaired due to a membrane damage brought about by PRL abnormality. This needs further clarification by way of morphological studies on Sertoli cells including EM studies.

Experimentally induced hyperprolactinaemia appears to arrest spermatogenesis during spermatid maturation. From the morphological studies, the extent of arrest and tissue damage appears to be dependent on the level of serum PRL level. For example in those rats with very high PRL levels morphological findings show that there are far less spermatids (both the elongated and round forms) than in those rats with moderately high PRL levels. Morphological findings on the latter group show more spermatids (both the round and elongated forms). The rats with very high PRL levels also have markedly lowered sperms within the lumen of the seminiferous tubules, the latter group also exhibiting a

reduction in cellularity and the presence of more cellular debris within the lumen. This is illustrated in figures 10 and 11 and in figure 6.

The above findings in the hyperprolactinaemic rats together with the appearance of cellular debris seen in testicular tissue suggest that hyperprolactinaemia impairs spermatogenesis in a dose dependent manner in the male. The possible site of damage is likely to be the Sertoli cells. In conclusion it could be said that there appears to be a definite effect of serum PRL both on the Sertoli cells of the testis. These findings need further studies in order to ascertain the molecular level at which PRL affects these sites. It could also be concluded that in addition to the findings of Charreau et al. [4] and Negro-Vilar et al. [5] according to this study prolactin exerts a direct effect both on the Sertoli cells and on the epididymal epithelium.

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