

Morphological Changes in the Epididymal Tissue of White Rats with Hypoprolactinemia

R. Hasan

Senior Lecturer, Department of Anatomy, Faculty of Medical Sciences, University of Kelaniya

Abstract— Male factor infertility due to endocrine disturbance such as abnormalities in prolactin levels are encountered in a significant proportion. This case control study was carried out to determine the effects of prolactin on the morphology of the male reproductive tract, using 100 male white rats belonging to the wistar strain. The rats were maintained as the control group (G1) and hypoprolactinaemic group (G2). After 100 days, the rats were subjected to serum prolactin (PRL) level measurements. The difference between serum PRL concentrations of rats in G2, as compared to the control group were highly significant by Student's *t*-test ($p < 0.001$).

Keywords— Male factor infertility, Hypoprolactinemia, Morphological changes in epididymal tissue, animal studies.

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]. Primary male factor infertility is when the man has never impregnated a woman whereas secondary male factor infertility is irrespective of the outcome of the pregnancy, man has impregnated a woman not necessarily the current partner [1]. It is publicized that infertility affects 10-15% of the world's population with two million new couples with infertility per year leading to immense psychosocial and personality disputes in most cases [2-5]. This implies the burden of the problem and the need to broaden the understanding of the pathophysiology of infertility in order to develop efficient intervention and treatment. The variety of causes of male factor infertility can be classified arbitrarily into pre-testicular, testicular and post testicular causes [6]. Other important causes are idiopathic spermatozoon abnormalities (40%), infection of the male accessory reproductive glands namely the prostate, seminal vesicles, iatrogenic insults to the testicles such as testicular irradiation, antimetabolic medication, androgen therapy, use of anabolic steroids and antihypertensive medications, antibiotics, and antipsychotic medication and autoimmune causes [7]. A link between male factor infertility and low sperm count has been described but the exact mechanism of which is unknown [7].

The pre-testicular causes account for up to 10% of male factor infertility and mainly include hormonal factors. This signifies the role of 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 [7]. The hypothalamo-pituitary hypofunction contributes to about 1% of cases [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 [8]. The role of PRL on the morphology of the male reproductive system 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 reduced prolactin level in the blood on the morphology of epididymal tissue in otherwise normal rats and thereby to determine whether hypoprolactinaemia 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 made of pellets obtained from Messers Moosajees Ltd, Colombo, 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. 100 rats were selected and grouped to G1 and G2. 50 rats were included in each group and maintained in separately labeled cages. These groups were subjected to the following procedures. Group 1 (G1) — The 50 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 50 rats in this group were fed with oral bromocriptine 4.65 mg per kg body weight per day in a divided dose twice a day, dissolved in 2ml 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. Hypoprolactinaemia was induced in the rats using bromocriptine. 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 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 sensitivity of 0.5ng/ml 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 male reproductive tract 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 using a plastic disposable syringe. The male reproductive tract of the rat was dissected and preserved in 10% formalin for 5 days. Sections of the epididymis were obtained using a new razor blade from each of the rats sacrificed. These sections were washed over night with tap water, labeled and tied up individually in small sacs made of surgical gauze.

III. RESULTS

The results of the morphological studies under light microscopy are illustrated in figures 1 to 4.



Fig. 1. Epididymal tissue of control rat.

Section prepared from the caput region of the epididymis. (H&E/LM X 132) Large tall cells in the pseudostratified epithelial lining are seen, as characteristic of the caput region. In addition, microvilli (stereocilia) which are borne by the PCs, are very prominently seen in the epithelial lining. The smooth muscle layer is also evident. The lumen on the left is filled with spermatozoa.



Fig. 2. Epididymal tissue of control rat.

Section prepared from the corpus region of the epididymis (H&E/LM x 132) The pseudostratified epithelial lining with the PCs bearing numerous microvilli. Also seen in this section are the basal cells. Note the presence of numerous spermatozoa (a) within the lumen.

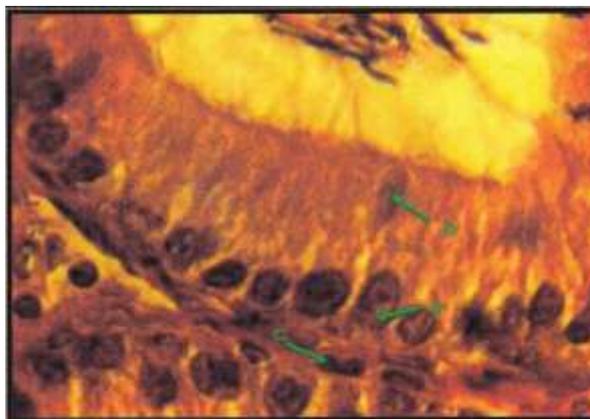


Fig. 3. Epididymal tissue of control rat.

Section prepared from the corpus region of the epididymis (H&E/LM x 330) The tall cells of the pseudostratified epithelial lining bearing numerous long microvilli. The PCs (a), apical cells (b) and basal cells (c) are clearly seen.



Fig. 4. Epididymal tissue of rat treated with oral bromocriptine.

Section prepared from the corpus of the epididymis (H&E/LM x 132) Pseudostratified epithelium is relatively devoid of microvilli. Spermatozoa are seen within the lumen

IV. DISCUSSION

From the results of the morphological studies carried out under light microscopy it appears that hypoprolactinaemia has no effect on the morphology of the epididymis or on the process of spermatogenesis.

From the results of the light microscopic morphological studies on the epididymis of both the rats with hypoprolactinaemia and also those in the control group it was evident that PRL had no notable effect on the epididymis except a relative reduction of the microvilli in the pseudostratified epithelium. The epididymis as has been noted earlier is merely a passage where secretions are added and serve as a conduit for the passage of sperms during ejaculation.

REFERENCES

- [1] P. J. Rowe, F. H. Comhaire, T. B. Hargreave, and H. J. Mellows (eds), "WHO manual for the standardized investigation and diagnosis of the infertile couple", Cambridge: Cambridge University Press, 1993.
- [2] P. J. Rowe and E. M. Vikhlyaeve, *Diagnosis and Treatment of Infertility*, Toronto: Hans Huner Publishers, 1988.
- [3] H. P. Azarian, T. S. Drampian, L. A. Tatevosian, "Main causes and frequency of male factor infertility in infertile couples," in *Diagnosis and treatment of infertility*. P. J. Rowe and E. M. Vikhlyaeva, Ed. Toronto: Hans Huner publishers. pp. 111-116, 1998.
- [4] G. Nowakowski, E. Widala, and D. A. A. Kochanska, "Hyperprolactinaemia: Aetiopathogenesis and clinical features," *Przegl Lek*, vol. 55, pp. 393-396, 1998.
- [5] A. Coppola, M. A. Cuomo, "Prolactinoma in the male. Physiopathological, clinical and therapeutic features," *Minerva Endocrinology*, vol. 23, pp. 7-16, 1998.
- [6] G. V. P. Chamberlain and T. L. T. Lewis, *Gynaecology by Ten Teachers*, Somerset: Great Britain, 1995.
- [7] D. M. Nudell, Male factor infertility and men's health, *Male infertility overview*, pp. 1-5, 2003.
- [8] A. Rogoza, W. Mierzejewski, and M. Puzio, "Detection and treatment of hyperprolactinaemia in male infertility," *Ginekaol Pol.*, vol. 65, pp. 75-79, 1994.