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EFFECT OF LH-RH ANALOGUE ON PITUITARY-GONADAL AXIS OF MALE ALBINO RAT

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ABSTRACT:
Lh-Rh analogues may give the antifertility action by acting on the Hypothalamus-Pituitary-Gonad axis that is either by changing the histology of normal reproductive tract, change in basal plasmatic concentrations of gonadotropins and by reducing the Lh receptors. Agonist analogs of Lh-Rh were originally developed as longer acting therapeutic agents to treat GnRH deficient patients. After early trials, it become apperant that chronic administration of these LHRH agonists, after a brief initial period of stimulation leads to paradoxical inhibition of gonadal functions in rodents, primates and humans. Lh-Rh agonist causing significant decrease in the sperm count ($P<0.01$) after 15 days as well as after 7 weeks also when compared with control. This may be due to deleterious effect of the drug on the Leydig cell that may consequently be responsible for testicular and epididymal dysfunction as a result of androgen deprivation. This may affect the process of sperm production and maturation in both organs leading to loss of fertility in treated rats.

Keywords:- Lh-Rh analogues, Hypothalamus, Pituitary-Gonadal axis, Testis, Epididymis and sperm count.

INTRODUCTION:

Release of all hormones from anterior pituitary gland are controlled by regulatory influence of central nervous system i.e. hypothalamus and called as hypothalamic hormones transmitted to the anterior pituitary via the portal system of capillaries in close contact with neurosecretory cells of the median eminence of the hypothalamus and draining to the anterior pituitary. There are six hypothalamic releasing and inhibiting hormones: corticotropin-releasing hormone (CRH); thyrotropin-releasing hormone (TRH); growth hormone (GH)-releasing hormone (GRH); Somatostatin (also known as growth hormone-inhibiting hormone (GHIH); GnRH; and dopamine (also called prolactin-inhibiting hormone) (McMahon *et al.*, 2001). These all releasing hormones are secreted by median eminance of the hypothalamus. **(Fig. 1)**

Hormones of Adenohypophysis

Functioning of testis is regulated by the two glycoproteins secreted by anterior pituitary that is FSH and LH. FSH and LH work together on the testis and plays different roles. Gonadotrophs comprise about 10% of the anterior pituitary cells. These cells contain small secretory granules. Gonadotrophs can produce LH, FSH or both hormones together. FSH plays a vital role in the maturation of gametes in males and females. LH stimulates testosterone secretion by Leydig cells in males and ovulation in the female. Gonadotropins (FSH and LH) are members of the glycoprotein hormone family. The pituitary glycoprotein hormones are derived from family that includes LH, FSH, TSH and placental chorionic gonadotropins (CG). Members of this glycoprotein hormone family are heterodimers, that is they are composed of two non-identical subunits designated α and β polypeptides. The pituitary glycoprotein hormones (FSH, LH and TSH) share a common α-subunit identical in structure. However, the β-subunits are unique to each gonadotropin and confer biological and immunological specificity to each hormone. The α and β-subunits of glycoprotein hormones are encoded by separate genes. There is a single gene

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for the α-subunit as well as the β-subunit of LH and FSH (Pierce and Parsons, 1981).

FSH acts by binding to specific receptors localized exclusively in the gonads. FSH receptor (FSHR) belongs to the family of G (guanine nucleotide-binding) protein-coupled receptors complex transmembrane proteins characterized by seven hydrophobic helices inserted into the plasma membrane and by intracellular and extracellular domains of variable dimensions depending on the type of ligands (Gudermann *et al.*, 1995). FSH and LH act on the gonads to regulate folliculogenesis, ovulation, spermatogenesis, and steroidogenesis. LH promotes the production of androgens (dehydroepiandrosterone, androstenedione and testosterone) from cholesterol. LH has a β-subunit of 121 amino acids that confers its specific biological actions and is responsible for interaction with the LHR. The structure, function and regulation of LH secretion have been well investigated in mammalian species (Nakav *et al.*, 2005). LHR is a member of the subfamily of glycoprotein hormone receptors within the superfamily of G protein-coupled receptors. LHR possess seven membrane-spanning domains or transmembrane helices (Dufau, 1998). In males, LH is also called as ICSH (Interstitial cell stimulating Hormone) which is responsible for stimulation of Leydig cells in the testis for the secretion of gonadal steroid, testosterone which plays pivotal role in the process of spermatogenesis.

Exponentially growing population has been adversely affecting the social, economical and technological development of human race. Contraception is important to health, development, and quality of life and has allowed couples to plan their families and safely space births. A good number of synthetic contraceptives are available in market, each one with either a limited success or side effects. Information regarding the drugs and formulations which may cause antifertlity in males is scanty. Several methods of contraception for family planning had been used over the years, however, nonreliability of these drugs in many cases impel us to investigate new formulations which can be used as an alternative synthetic contraceptives. LhRh analogues may give the antifertility action by acting on the Hypothalamus-Pituitary-Gonad axis that is either by changing the histology of normal reproductive tract, change in basal plasmatic concentrations of gonadotropins and by reducing the LH receptors. Normal testicular function requires stimulatory actions of pituitary gonadotropins LH and FSH. Luteinizing hormone stimulates Leydig cells stereoidogenesis to generate and maintain intratesticular concentrations, which are essential for initiating and maintaining spermatogenesis. Agonist analogs of LHRH were originally developed as longer acting therapeutic agents to treat GnRH deficient patients. After early trials , it become apperant that chronic administration of these LHRH agonists, after a brief initial period of stimulation leads to paradoxical inhibition of gonadal functions in rodents, primates and humans.

Thus this study aims to investigate following aspects regarding the Albino wistar rat *Rattus rattus.*

1. Effect on Histoarchitecture of testis and effect on the seminiferous tubules, Leydig cells and spermatogenesis after administration of LH-RH analogue (250 µgm).
2. Effect on basal plasmatic level and role played by gonadotropic hormones (FSH and LH) and male sex steroid testosterone after administration LH-RH analogues (250 µgm) after 15 days and 7 weeks.

MATERIALS AND METHODS

The albino rat Wistar strain, Rattus rattus is a species which belongs to the Muridae family of Muroidea superfamily and order Rodentia under

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the subclass Eutheria of mammalia is selected for the present study.

Animals: Adult male albino rats, Wistar strain weighing 1750-225 gm body weight used for the study were housed under standard laboratory condition (rooms are maintained at 30-70% relative humidity and a temperature of 18-26°C). They were fed with standard rodent pellet and water ad libitum. The animals were grouped in to two groups of 12 animals each for each experiment..

a. Control group and b. Experimental Group : Rats receiving different doses of LH-RH analogues. ExperimentNo:1

Three groups of 12, experimentally naive males, were reared and administered normal saline and LH-RH analogues (250 µgm) subcutaneously daily for 7 weeks. Six animals from both experimental and control group were sacrificed after 15 days whereas remaining animals were sacrificed at the end of 7 weeks. (Table 1)

Hormonal assay: Blood samples from anesthetized rate were collected from the cardiac puncture was collected in heparinised capillary tube. Blood samples were centrifuged for 10 minutes in a centrifuge. A supernatant plasma was collected into micro centrifuge tubes and frozen at -20 0C until assay. LH, FSH and testosterone were analysed using RIA kits. Data were expressed in the form of mean ± standard error. ANOVA, Tucky HSD test, correlation matrix, correlation coefficients and regression equations.

LH: VIDAS LH is the automated quantitative test for use on the VIDAS instruments for enzyme immunoassay determination of LH in serum or plasma using the ELFA technique.

FSH:VIDAS FSH is the automated quantitative test for use on the vidas instruments for enzyme immunoassay determination of FSH in serum or plasma using the elfa technique.

Testosterone: VIDAS testosterone is the automated quantitative test for use on the VIDAS instruments for enzyme immunoassay determination of testosterone in serum or plasma using the ELFA technique.

Sperm count: Sperm count were assessed in cauda epididymis by the standard methodology.

Light Microscopy (Histological Staining Methods) for Histopatlological Examination.

Animals under experimentation were anesthetized with the anesthesia ether. The reproductive tract was dissected out from the sacrificed animal and was freed from extraneous fat and Pituitary, testis, thyroid, and adrenal gland were fixed in aqueous Bouin's fixative for 24 hours and then washed with 70 % ethanol. The tissues dehydrated in 80%, 90% and absolute alcohol were cleared in xylene and embedded in paraffin wax. The embedded material was sectioned serially at 4 - 6 µm thickness. Sections were stained with hematoxylin-eosin staining technique (Humason, 1979). Microphotographs of histological sections were captured using Labomade DG-3 compound microscope camera at 100X and 400X magnification. Tagged image file format was used to measure the changes at histological level.

Statistical analysis: Mean,Standard error, Standard deviation, Variance, ANOVA with post hoc Tucky with HSD, are calculated by using Statistical Package for Social Sciences (SPSS 10.0).

OBSERVATIONS AND RESULTS:

Gonadotropin Hormones and Testesterone Assay in LHRH analogue treated and Control Albino rats: Studies assessed the effect of 250 µgm dose of LHRH analogue, D-Ser-(TBu)6-EA10-LHRH, agonist on LH, FSH and Testosterone concentrations. Daily administration of this agonist resulted in an early phase of stimulation, followed by a progressive decline in serum LH, FSH and testosterone concentrations to the serum below baseline by the 15th day of treatment. The 250 µgm dose of

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LHRH analogue was more potent in both stimulatory and down regulatory effects (**Table. 2 & Fig.2).**

Effect of LHRH agonist on Sperm Count of albino rat : During present investigation there was a increase in sperm count in LHRH agonist treated rats (Subcutaneous Injection 250 µgm daily) compared to the control rats after 15 days. However this phase was not constant and there was significant decrease in the sperm count after 7 weeks of treatment with LHRH agonist. In This study spermatogonia cells, primary spermatocyte, spermatid and spermatozoa decreased significantly in both right and left testes of the experimental group compared to control group (P<0.01). (**Table 3 & Fig. 3)**

Histopathological effect of LHRH agonist on Testes: In this study transverse section of control rat's testis showed presence of seminiferous tubules held together by connective tissues that contains blood vessels and nerves and muscle fibres. Connective tisssues have an interstitial cell that secretes testosterone. Each seminiferous tubule showed developmental stages of sperm, which are spermatogonia cells, primary spermatocyte, spermatid and spermatozoa (Fig.4). Daily administration of LHRH agonist shows significant destruction of the process of spermatogenesis due to the destruction in the germinal epithelial cells and inhibitory effect on the pituitary-gonadal axis which results in the significant decrease in the testosterone concentration which is key factor in the process of spermatogenesis (**Fig.5).**

Histopathological effect of LHRH agonist on Epididymis: Transverse section of control epididymis showed compactly packed tubules, lined with well defined pseudostratified epithelium (Figs*:6*). LHRH agonist has caused the damage to the epithelial lining of the epididymal tubules, increase in the interluminal area as well as increase in the lumen present within the epididymal tub*u*les (Figs*:7*). Significant decrease in the spermatogenesis resulted in the loss of spermatic elements in the epididymal tubules.*(Fig:8)*

CONCLUSION:

Daily subcutaneous injection of Lh-Rh analogue (250µgm) for 15 days and 7 weeks resulted in atrophy of the testes and impairment of fertility. The drug have antifertility potency when the animal is exposed to these drugs for longer duration. Subcutaneous administration of these drug (250µgm) daily for 15 days was sufficient to cause the deleterious effect on the pituitary-gonadal axis. Lh-Rh agonist treatment causes a marked reduction in the steroidogenesis which results in the significant decrease in the concentration of testosterone responsible for infertitility in males. LHRH agonist resulted in the early stimulatory effect on the gonadotrophs and progressive decrease in the synthesis and secretion of GhRH. Thus showing both stimulatory and down regulatory effect on the gonadotrophs. This results in the early phase of increase in the level of concentrations of LH, FSH and testosterone followed by significant decrease in their concentrations (P<0.01). LHRH agonist causing significant decrease in the sperm count (P<0.01) after 15 days as well as after 7 weeks also when compared with control. This may be due to deleterious effect of the drug on the Leydig cell that may consequently be responsible for testicular and epididymal dysfunction as a result of androgen deprivation. This may affect the process of sperm production and maturation in both organs leading to loss of fertility in treated rats.

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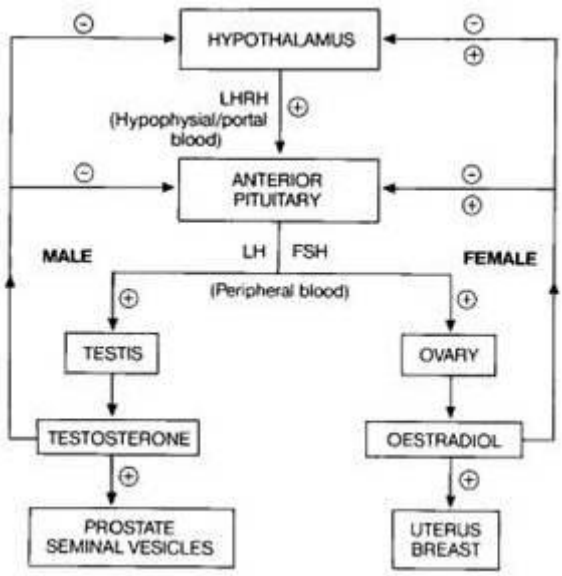

Fig.1 Showing Hypothalamic- Pituitary-Gonadal axis and its feedback mechanism

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Table 1: Protocol for Experiment No. 1

No. of Male Rats	Drug and dose	Route	Duration
12 Control Group	Vehicle (Equal Volume)	Subcutaneous	15 days and 7 Weeks
12 Expt. Group	LH-RH analogues (250 µgm)	Subcutaneous	15 days and 7 Weeks

Table: 2. Effect of LHRH agonist administration on Mean plasma FSH, LH and testosterone levels in male albino rats.

Group	Days After LHRH agonist (250 µgm) administration	FSH (mIU/L)	LH (IU/L)	Testosterone (ng/ml)
Control	15 days	3.4 ±0.23[a]	3.1±0.18[a]	9.2±0.7[a]
Experimental Group	15 days	4.4 ±0.15[a]	3.8±0.12[a]	10.3±0.8[a]
Control	7 Weeks	3.7±0.17[a]	2.9±0.29[a]	8.8±0.6[a]
Experimental Group	7 Weeks	1.8±0.16[b]	1.6±0.12[b]	3.7±0.85[b]

Note: Within column, Mean ± S.E. with the same superscripts are not significantly different at (P < 0.01).

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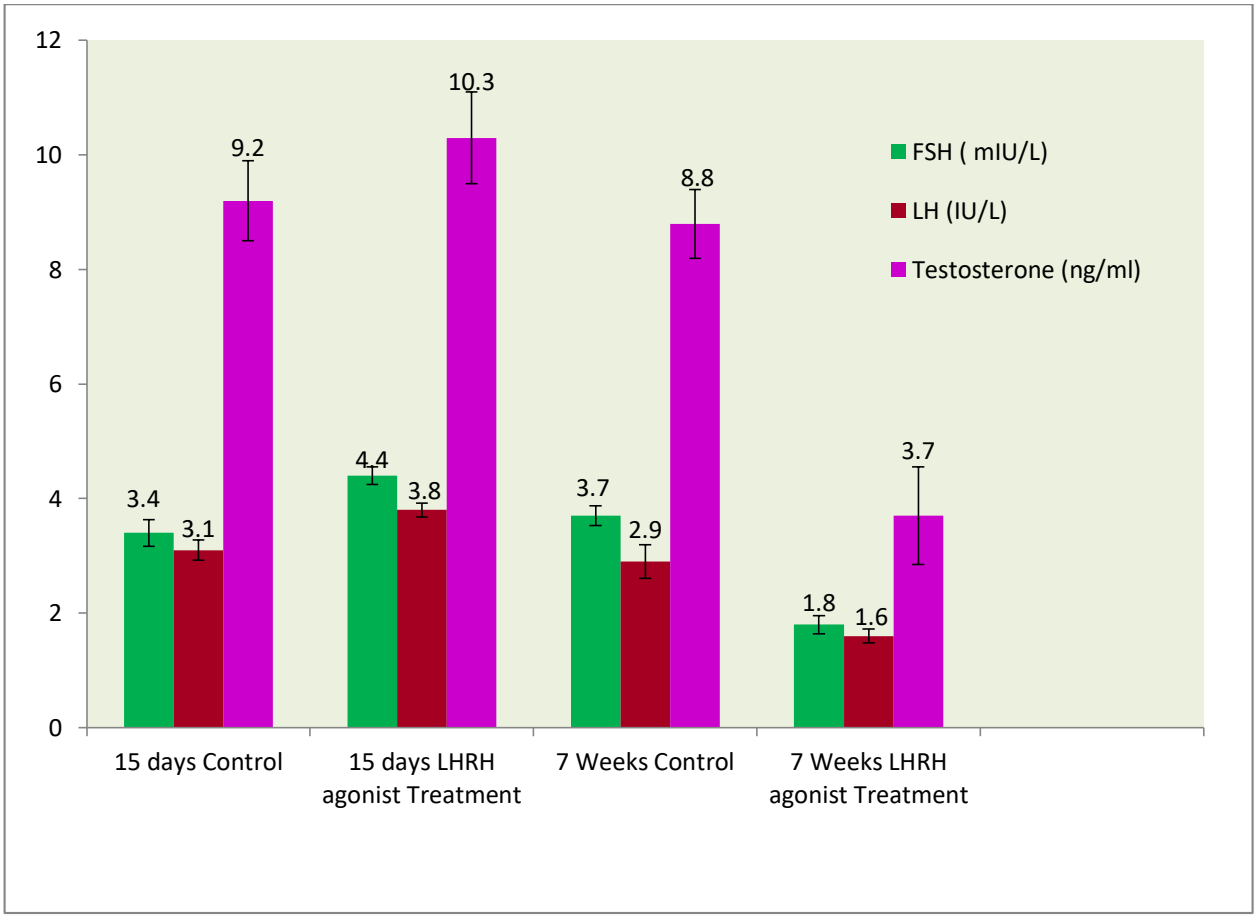

Fig: 2 Graphic representation of effect of LHRH agonist administration on Mean plasma FSH, LH and testosterone levels in male albino rats after 15 days and after 7 weeks.

Table: 3. Effect of LHRH agonist administration on Mean sperm count in male albino rats.

Group	Days After LHRH agonist administration	Mean sperm count in Millions/ml
Control	15 days	82.6 ±1.58[a]
Experimental Group	15 days	86.4 ±1.72[a]
Control	7 Weeks	81.2±0.97[a]
Experimental Group	7 Weeks	27.8 ±1.42[b]

Note: Within column, Mean ± S.E. with the same superscripts are not significantly different at (P < 0.01).

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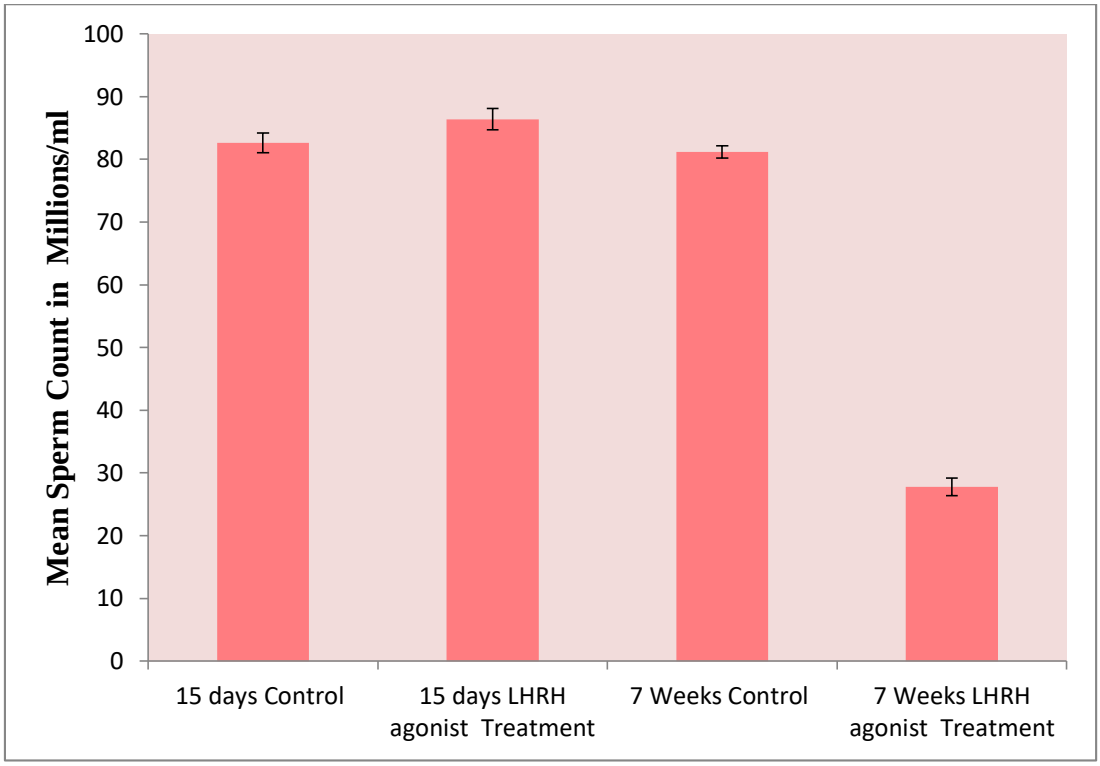

Fig:3 Graphic representation of effect of LHRH agonist administration on Mean sperm count in male albino rats after 15 days and after 7 weeks.

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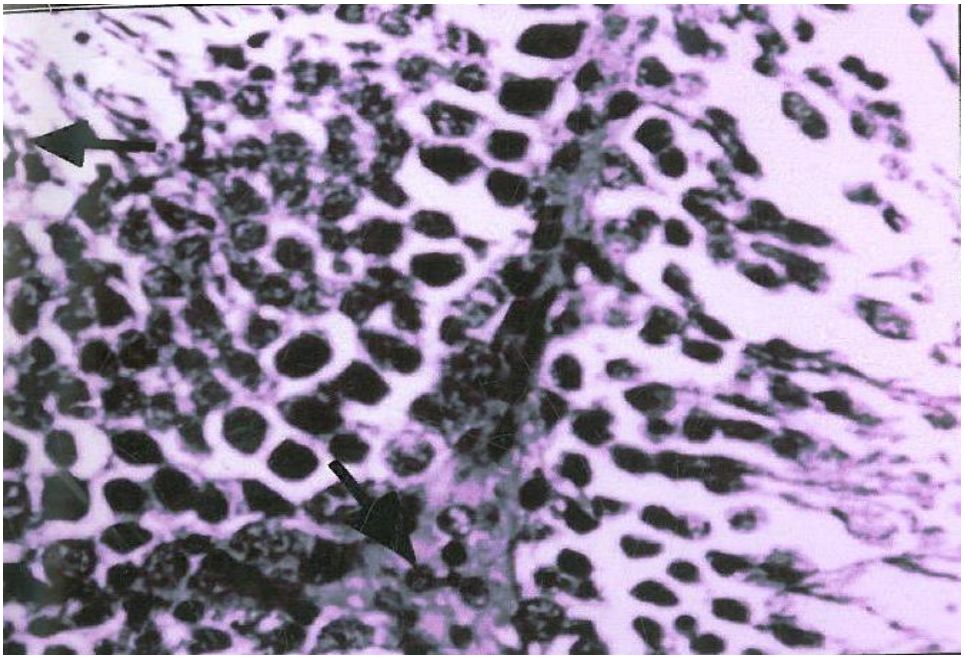

Figure:4 Transverse Section through normal testicular tissue of control group of Wistar rats showing normal spermatogenesis. (HE x100)

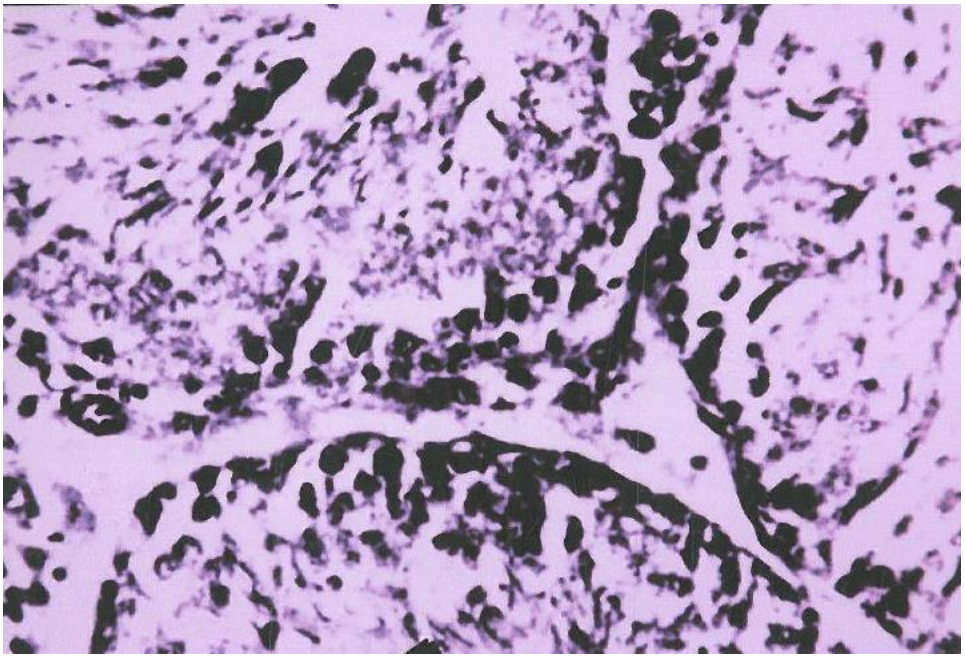

Figure: 5 Transverse Section through testicular tissue of Experimental group of Wistar rats treated with LHRH agonist for 7 weeks showing degenerative changes in the seminiferous tubules. (HE x100).

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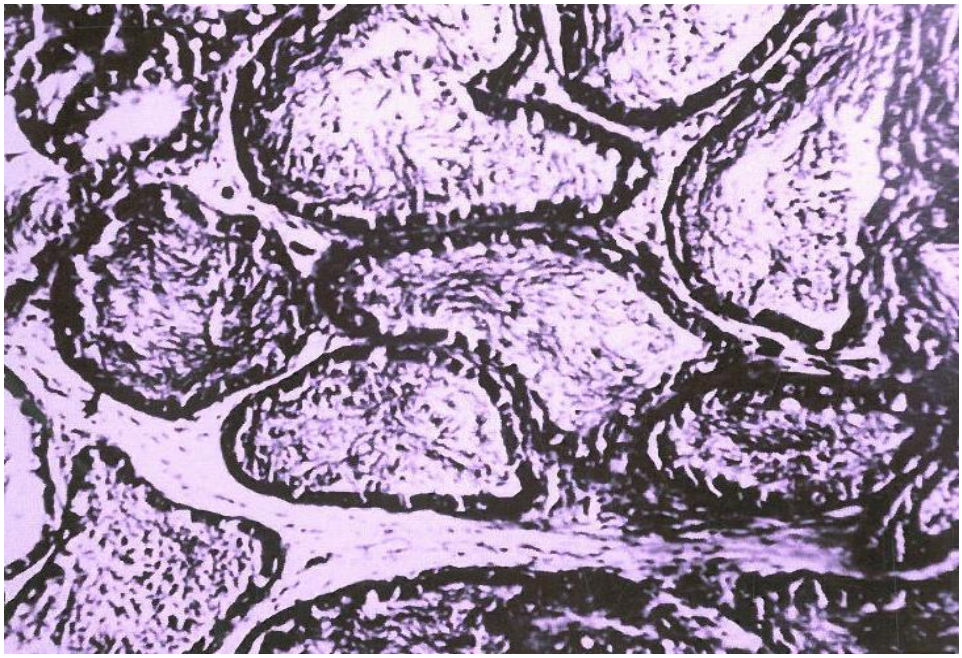

Figure: 6. Transverse Section through Caput Epididymis of control Wistar rats showing normal histology. (HE x100).

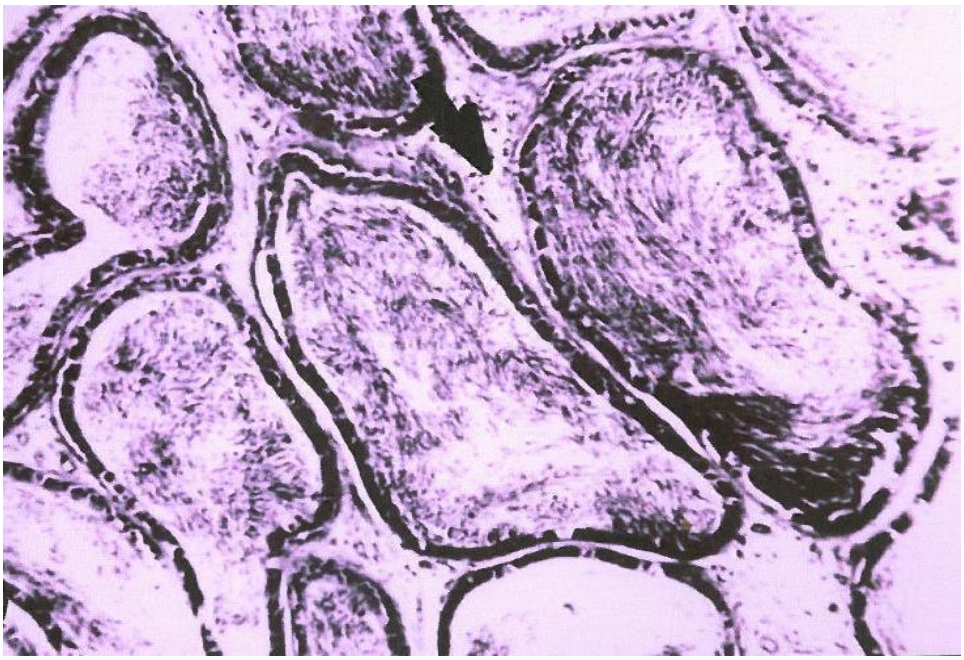

Figure:7. Transverse Section through Caput Epididymis of LHRH agonist treated Wistar rats showing decrease in epithelial height, increase in interlobular area and loss of spermatic elements . (HE x100).

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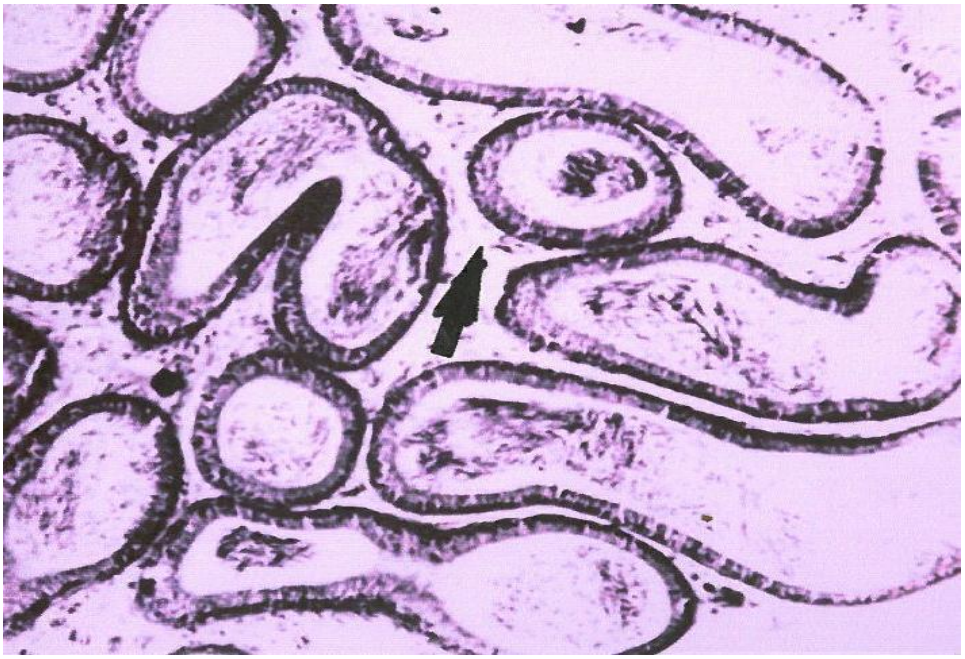

Figure: 8. Transverse Section through Cauda Epididymis of LHRH agonist treated Wistar rats showing decrease in epithelial height, distortion of normal histology and loss of spermatic elements . (HE x100).

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