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EFFECTS OF NORISTERAT ON FERTILITY AND OVARIAN-PITUITARY AXIS IN A FEMALE PALM SQUIRREL *FUNAMBULUS PENNANT* (WROUGHTON)

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ABSTRACT:
Noristerat (Norethisterone Enanthate, NET-En) is used for the present study. This drug inhibits ovulation due to suppression of GnRH and pituitary gonadotrophins FSH & LH. On the basis of pharmacological – endocrinological background, these compounds have been used for fertility control in female. Noristerat has multiple site of action, as well as its primary site of action one still controversial. Considerable disagreement exists regarding the action of NET-En on anterior pituitary cell types. The paucity of information has led to reexamine this compound. The experiment was conducted on female palm squirrel during July-August i.e. at the end of breeding season. Female squirrels of experimental group received 0.5 mg/kg. B.W./week Noristerat for one month. Noristerat treated animals showed retarded growth of Graafian follicles. Except primary follicles other follicles showed degeneration. These degenerations were more evident in granulosa cells and oocytes. Ovary contained several atretic follicles. Corpus luteum disappeared from the ovary. Noristerat treated anterior pituitary showed no changes in growth hormone producing cells, but induced regressive changes such as reduction in number and size of cells & degranulation of FSH, LH & PRL cells.
Direct action of Noristerat on ovary can be ruled out because if we assume that the Noristerat directly act on ovary and regress Graafian follicle and inhibit estrogen. Deficiency of estrogen expect hyperactivity of FSH cells which do not occur in the female squirrel. Deficiency of estrogen is responsible for the suppression of ovulation.

Keywords : *Ovarian-pituitary axis, Noristerat.*

INTRODUCTION:

Noristerat (Norethisterone Enanthate, NET-eN) is an ester of norethisterone and in a long acting intramuscular depot contraceptine drug. It is fully reversible. This contraceptive inhibits ovulation due to suppression of GnRH and pituitary gonadotrophins FSH & LH (Greenwald, 1965; Faundes *et al*, 1991; Emones *et al* 1992). This contraceptive has some positive side effects such as it protects against ovarian and endometrial cancer and insignificant yet undesirable side effects such as nausea, head ache and break through bleeding. The manner in which NET-En acts as a contraceptive drug as well as its primary site of action are still controversial. Considerable disagreement exists regarding the action of NET-En on anterior pituitary cell types. The paucity of information has led to reexamine this compound.

METHOD AND MATERIAL:

Noristerat was purchased from the local chemist (manufactured by Schering AG Berlin, Germany). The experiment was conducted on female palm squirrel during July-August i.e. at the end of breeding season.

The detail experimental design, grouping and protocol given in the Table 1. Female squirrels of experimental group received 0.5mg/kg. B.W./week for 1 month. Noristerat was diluted in arachis oil and control received equivalent volume of vehicle.

Pituitary sections were stained with Crossmon's Triple stain and PAS/MB/OG staining techniques. Staining techniques for cell types of Anterior pituitary and their tinctorial properties are mentioned in Table-2.

RESULTS AND DISCUSSION

Ovary of Control Female (Fig. 1 & 2)

Ovary showed peripheral layer of germinal epithelium. Stroma was quite thick, composed of dense mass of connective tissue fibres. Stroma contained follicles in various stages of development. Antral Graafian follicles were present in the ovary. Ooyte was surrounded by zona pellucidea which was covered by corona radiata. Normal interstitial cells and regressed luteal cells were present in the stroma.

Ovary of Experimental Female (Fig. 3, 4, & 5)

Noristerat (0.5 mg/kg. B.W./week for one month) treated animals showed retarded growth of Graafian follicles. Except primary follicles other follicles showed degeneration. These degenerations were more evident in granulosa cells and oocytes. Ovary contained several atretic follicles. Corpus luteum disappeared from the ovary.

Noristerat, a derivative of 19-nortestosterone. It is synthetic injectable progestogen contraceptive suitable for women who cannot take oral contraceptives regularly.

Inhibition of growth of primary follicles and degeneration of secondary follicles in the ovary of palm squirrel following Noristerat treatment is similar to those of previous investigators who undertook clinical investigation in women and laboratory animals, (Wilks *et al*, 1983; Gupta *et al*, 1984; Grubb *et al* 1989).

Inhibition of growth of primary follicle and degeneration of secondary follicle may be due to insufficient production of FSH and LH hormones by the regressed FSH and LH cells of pituitary gland. Gonadotrophin hormone FSH and LH stimulate growth and maturation of Graafian follicle.

Very few investigators reported the effects of synthetic progestin on pituitary gonadotrophin cells. Present investigation on palm squirrel reveals that Noristerat treatment regressed FSH, LH & PRL cells of anterior pituitary gland in female. Similar regressive changes in the pituitary gonadotrophin cell have been reported by other investigators (Mendoza *et al*, 1993).

Synthetic progestogens suppress gonadotrophic hormone (FSH & LH) level in plasma (Garza-Floris *et al*, 1991; Cahill *et al*, 1996).

There are differences of opinion concerning effects of Noristerat on prolactin (PRL) cells of pituitary gland. In present investigation on squirrel Noristerat induce regressive changes in the prolactin cells. It has been well established that prolactin is considered as a gonadotrophic hormone in non-primate animals, where as prolactin have not been considered as gonadotrophic hormone in primates and human.

Mechanism of Antifertility Action of Noristerat

The precise sites and mechanism of antifertility action of Noristerat in female is still obscured. Various sites and mechanism of action have been proposed to explain its antifertility action. This drug exerts its antifertility action at hypothalamus and pituitary level by inhibiting GnRH and gonadotrophin (FSH and LH) cells thereby inhibiting ovulation in female (Garza-Floris, *et al* 1991; Mendoza *et al*, 1993).

Present study showed that intramuscular injection of Noristerat induce inhibition and degeneration of Graafian follicle is presumably mediated by the inhibition of gonadotrophin (FSH, LH and probably PRL) stimulation of ovary. Formation and development of Graafian follicles are under the control of FSH, LH and PRL. PRL is the inhibiter of ovulation therefore low secretion of FSH, LH and PRL due to Noristerat may account for the ovarian regression.

Direct action of Noristerat on ovary can be ruled out because if we assume that the Noristerat directly act on ovary and regress Graafian follicle and inhibit estrogen. Deficiency of estrogen expect hyperactivity of FSH cells which do not occur in the female squirrel.

Therefore it is concluded that the action of Noristerat on ovary is not direct but via anterior pituitary gland.

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Table 1: Experimental Design for Noristerat

Number of Animals & sex	Treatment	Dose mg/kg. B.W.	Administration	Duration (Months)
10 Females (Experimental)	Noristerat	0.5mg/week	I.M.	1
10 Females (Control)	Arachis Oil (Vehicle)	E.V. (0.1 ml)	I.M.	1

E.V. = Equivalent Volume I.M.= Intramuscular (injection)

Table 2: Staining techniques and cell types of Anterior pituitary and their tinctorial properties.

Staining Techniques	Cell Types					
	Type I GH	Type II PRL	Type III TSH	Type IV FSH	Type V LH	Type VI ACTH
Crossmon's triple stain Crossman (1937)	Yellow	Red	Dark Blue	Blue	Light Blue	±
PAS/MB/OG Wilson & Ezrin (1954)	Yellow	Yellow	Pink	Pinkish Blue	Pink	±

± Not specific

Plate 1 : Effects of Noristerat on ovary of *Funambulus pennanti*
Sections are stained with Haematoxylin and Eosin (HE)

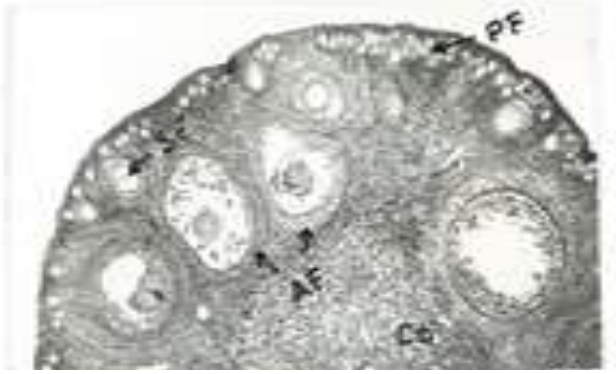

Fig. 1: Control ovary showing various stages of Graafian follicles i.e. primary, secondary and antral follicles. All follicles were normal. Cortex composed of degenerated luteal cells and connective tissue. (×20)

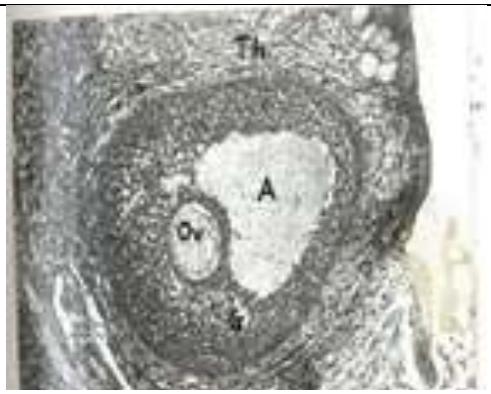

Fig. 2: Control Enlarged antral follicle showing normal histological structure (×115)

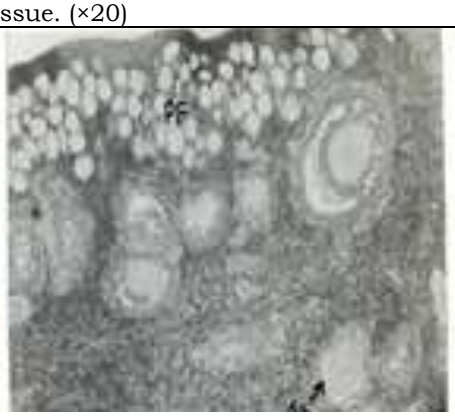

Fig. 3: Expt. ovary showing normal primary follicles (×115)

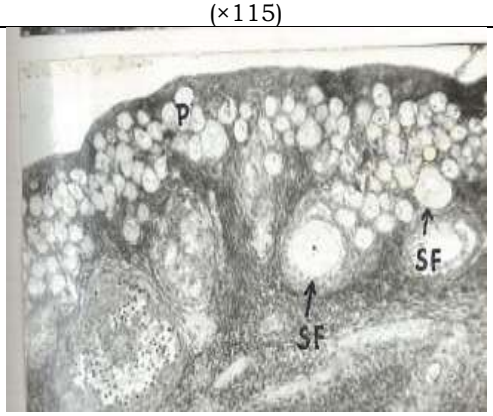

Fig. 4: Expt. ovary showing degenerating secondary Antral follicles & atretic follicles (×115)

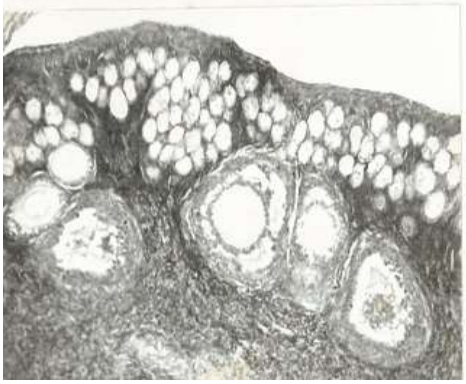

Fig. 5: Expt. ovary showing degenerative changes in oocytes and granulosa cells of multilaminar Graafian follicle (×115)

Plate II : Effects of Noristerat an Anterior pituitary of female palm squirrel *Funambulus pennanti* Sections are stained with Crossman's triple stain. (×1400).

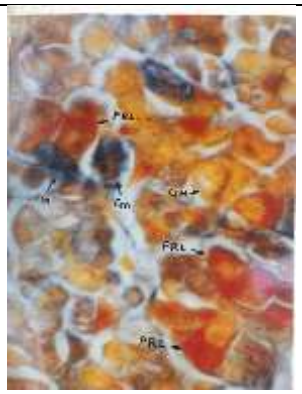

Fig. 6: Control Anterior pituitary showing GH (yellow) PRL (Red) and Gn (Blue) cells (Gn-Gonadotraphin cells)

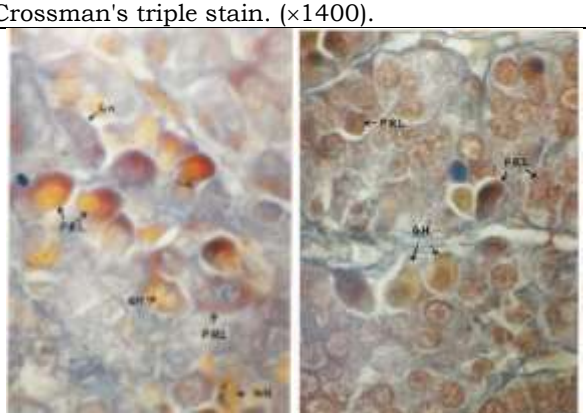

Fig. 7: Experiment Anterior Pituitary showing unchanged GH cells and regressed PRL and Gn cells

Sections of Anterior pituitary stained with PAS/MB/04 (X1400)

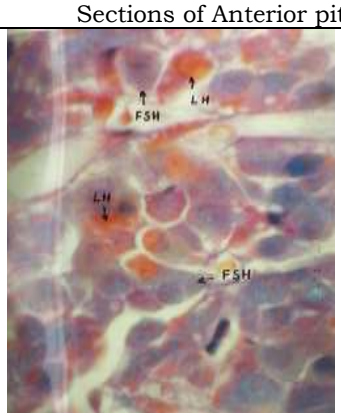

Fig. 9: Control Anterior pituitary showing normal FSH (pinkish-blue) and LH (pink) cells.

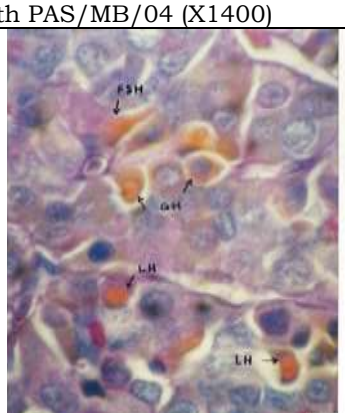

Fig. 10: Expt. Anterior pituitary showing regressed FSH & LH cells.