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To study the effect of norethisterone as a hormonal contraceptive on the ovary of albino rat (Wistar Strain): Histo-clinical approach

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Abstract
Noristerat or norethisterone or norethindrone or heptanoate is a depot progestogen for hormonal contraception. Synthetic progestin (NET) exerted pharmacological effects at multiple levels of both the hypothalamic pituitary- Ovarian axis and the female genital tract. Norethisterone heptanoate was the first synthetic progestational agent which was popularly used as a long acting injectable contraceptive. synthetic progestins may have direct effect on ovary by influencing the LH and FSH ratio.

Keywords: NET, Noristerat, Norethisterone heptanoate, ovary, Pituitary, FSH, LH, hormonal contraception.

Introduction

Norethisterone

Norethisterone was the first highly active oral progestational agent to be synthesized and to achieve wide spread use along with its acetate and enanthate esters.

Chemistry: Norethisterone (norethindrone) C20 H26 O2**:** 170- Ehinyl-19-nortestosterone, 17B-hydroxy-19-nor-170-pregn-4-en-20-yn-3-one, 170-ethinyl-17B-hydroxy-19-nor-androst-4-en-3-one.

Norethisterone Heptanoate

Noristerat or norethisterone or norethindrone or heptanoate is a depot progestogen for hormonal contraception, Engren, (1974) [20] have defined norethisterone as an androgen with a typical progestational effect at sufficiently higher doses. Kobra *et al*. (1974) [25] observed that fertility was not adversely affected even after long period of norethisterone treatment. Howard *et al*. (1975) [10] suggested that norethisterone is supposed to exerted its antifertility effect. Erik Weiner *et al*. (1975) [6] concluded that norethindrone enanthate inhibited ovulation. Howard *et al*. (1985) [11] suggested that norethisterone heptanoate was the first synthetic progestational agent which was popularly used as a long acting injectable contraceptive. According to Chang *et al*. (1984) [4] and Veldhius *et al*.; (1989) [23] the synthetic progestins may have direct effect on ovary by influencing the LH and FSH ratio. Kim *et al*. (1991) [13] reported suppression of ovarian activity and atrophy of endometrium in women treated with norethisterone. Corpus luteum, endometrium, fallopian tubes were reacted independently after norethisterone treatment.

Poindexter III *et al*. (1993) [2] found that the synthetic progestin (NET) exerted pharmacological effects at multiple levels of both the hypothalamic pituitory- Ovarian axis and the female genital tract. Banerjee *et al*. (1986) [3] studied the return of fertility following discontinuation of norethisterone oenanthate (NET-EN), in the same year Song Si (1993) [21] reported that the main action of norethisterone visiting pill, when started during that early phase of the cycle is suppression of cervical mucus and endometrial function.

Song *et al*. (1993) [21] studied the effects of different doses of norethisterone on ovarian function, hormone binding globulin and high density lipoprotein cholesterol. Laurikk- Routti *et al*. (1992) [17] observed suppression of ovarion function transdermally administered synthetic progestin ST 1435.

Materials and Methods

Young, healthy, sexually mature female albino rats of Wistar strain (120-150 gms body weight) with normal reproductive history were procured from Haffkine Biopharmaceuticals. The animals were kept under uncontrolled room ambient temperature and photoperiod. Food pellets marketed by Lipton India Limited and water provided ad libitum. The rats were acclimatized for a month to the laboratory conditions prior to the commencement of any experiment. Animals were divided into two sets as control and for drug treatment, for each set of an experiment a population of female rats belonging closely to a certain weight group were selected, the reason for which all the groups of rats at the commencement of the treatment did not weigh the same.

The animals were divided into control and experimental groups. The treatment lasted for 24 weeks duration i.e 24 injection of i.m. injectable norethisterone heptanoate, est. The drug were of 100% purity which is available in the market with same trade name.

On the completion of the treatment period, the animals were weighed and sacrificed under light ether anesthesia. The ovaries was quickly excised cleared off the adhering fat blotted and weighed after which processed for the various light and biochemical studies.

Observations and Discussions

Control Ovary

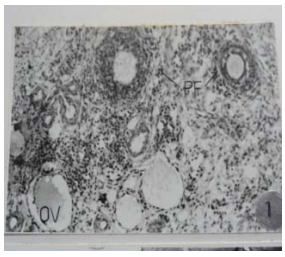

Fig 1: control ovary section showing the cortex, the germinal epithelium& primary follicles (PF) in various stages of developments (X- 40)

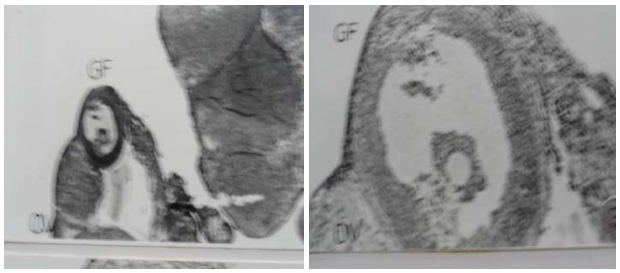

Fig 2&3: Showing growing follicle (GF), Ovum, & corpus luteum (X- 40) (X- 120).

Control Ovary

Histologically the structure of rat ovary is as follows.

Germinal epithelium

It is composed partly of flat and partly cuboidal cells. The flat cells have fusiform or spindle shaped nuclei while the cuboidal cells of the germinal epithelium tend to be flat in those places, where there is a large follicle or a corpus luteum to it.

Tunica albuginea

It varies in its thickness in different regions of the ovary and is being composed of many layers of fusiform cells. These cell contain oblong darkly staining nuclei. The connective tissues in this region are either straight or wavy.

Ovarian stroma

Stroma is composed of fusiform cells having spindle or ovoid nuclei and irregularly disposed connective tissue fibres. A few interstitial cells also occur in the stroma. The stroma is supplied with a few blood vessels. The following types of follicles are present in the ovary.

Primordial follicles: Are the youngest of the follicle and are apparently the cells of the germinal epithelium which sink deeper and get surrounded by a few cells, the satellite cells, the nucleus of the oocyte is eccentric in the majority of the primordial follicles. The cytoplasm is homogenous to granular.

Unilaminar follicles: Are formed by an increase in the size of the primordial follicle. The follicle cells remain cuboidal and finally columnar in the late unilaminar follicles. The nuclei occupy basal position leaving larger areas of cytoplasm towards the ovum. The theca follicle is one cell thick and is composed of fusiform cells and fine connective tissue fibres. The nucleus of the oocyte has increased diameter over that at the primordial stage. The cytoplasm of oocyte is granular (fig.1).

Bilaminar follicle: The ovum gets surrounded by two layers of follicular cells made up of columnar cells, the nuclei of the follicle cell are round to oval (fig.1).The Nucleus of the oocyte occupies an eccentric position in the majority of the bilaminar follicles but it may be central in few cases. There is an increase in the size of both the oocyte and its nucleus. The granular cytoplasm is more distinct. The zona pellucida is increased in thickness and may be homogeneous. The theca becomes two to three layered at this stage.

Multilaminar follicle: containes six to eight layers of follicle cells. These cells are polygonal in shape with spherical and ovoid, with a large and more or less centrally placed nuclei. The zona pellucida is thickned (fig.1), the cytoplasm of the oocyte is vacuolated and the cytoplasmic granules are arranged in the form of a reticulum. The cells of the theca are fusiform and there is no demarkation between theca externa and theca interna. Blood capillaries are mostly parallel to the surface of the follicle, present in theca.

Growing follicle or Vesicular follicle

The intercellular spaces are enlarged progressively by the accumulation of fluid and the cells are pushed further apart from each other. The large antral cavity is thus formed in the follicle. There is no appreciable increase in the size of the oocyte as compared with the size of the oocyte in the multilaminar follicle. The follicle is made up of five to six layers of fusiform cells and connective tissue fibres. There is no as such demarkation between theca externa and interna (fig.2, 3).

Norethisterone Treated Ovary

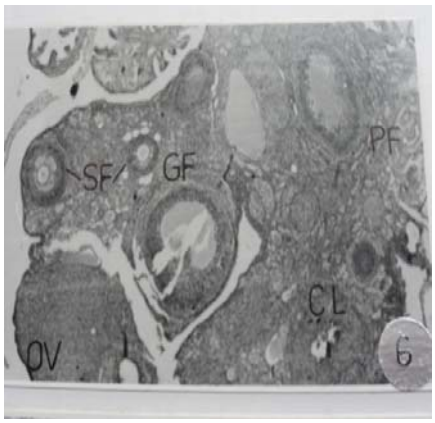

Fig 4: Section through ovary after theca Norethisterone heptanoate treatment 24 weeks, showing primary follicle(PF), secondary follicle(SF), Growing follicle(GF), aretric primary & multilaminar follicle, Young and slightly regressed corpus luteum. (X-40)

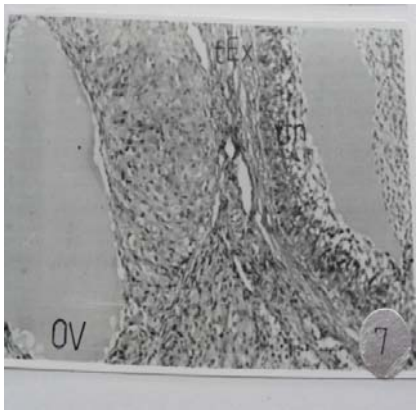

Fig 5: Magnified partoif atretic unilaminar follicle showing theca interna and theca externa. (X-120)

Ovary with norethisterone treatment does not undergo any change in its germinal epithelium, tunica albuginea and ovarian stroma as compared to the control specimen (fig.6).

The multilaminar follicles in the treated ovary contained six to eight layers of polygonal follicle cells. These cells are large with a spherical centrally placed nuclei as in normal specimen and vacuolated cytoplasm and reticulated granules are similar to that of control specimen (fig. 6).

The appearance of vesicular follicle is same. A corpus luteum is slightly regressed in stage and young corpus luteum is usual finding (fig. 6). There is slight follicular atresia by norethisterone treatment (Fig.7).

The mechanism of contraceptive action of norethisterone heptanoate is elucidated by investigating its effects on various target organs.

Extensive studies have been worked out in humans in relation to the effect of norethisterone on gonadotropins and its effect on ovarian function. (Larsson and Cohn *et al*. (1970b) [14], Moghissi *et al*. (1973) [19], Feldman (1973) [27], Fotherby *et al*. (1974, 1978) [7], Elstein (1976) [5], Landgren *et al*. (1990) [26], Anand kumar (1991) [1].

In the present study the treatment of norethisterone heptanoate to albino rat for 24 weeks revealed the presence of primary follicles, secondary follicle, multilaminar follicle, vesicular follicle and presence of slightly regressed corpus luteum.

Hormonal Assay

Table 1: X ± SEM Value

Sr. No.	Parameter	Control Value X1(5)	Treated Value X2 (5)
1	FSH MIU/ml	4.06 ± 0.085	6.63 +1.66
2	LH MIU/M/ml	3.36 ± 0.21	3.95 ± 0.02
3	Oestradiol pg/ml	158.64 ± 60.05	36.95 ± 16.76
4	Progesterone ng/ml	20.84 ± 2.98	15* ± 2.24
5	17(OH) Prog. Ng/ml	1.424 ± 0.223	0.905* ± 0.103

The hormonal assay in the present study reveals slight increased level of FSH but no changes in LH level. It is possible however that under sustained levels of FSH and LH, several follicles may reach maturation without rupture as reported by Moghissi and Mark (1971) [18].

In the present study the presence of growing follicle revealed the normal maturation process but this follicle may not rupture nor it may undergo further maturation for ovulation, because serum estrogen level registered a decrease. Regressed corpus luteum will lead to decrease in progesterone. Our results support the conclusion drawn by Anand kumar (1991) [1], who commented that norethisterone prevented follicular rupture and ovulation due to alteration of gonadotropins. It seemed reasonable to postulate that in norethisterone treatment some FSH activity persisted in this situation, conditioning the follicular growth. Similar results were also evidenced by Landgren *et al*. (1990) [26], reported that treatment of norethisterone for 3 months altered the FSH, LH and plasma progesterone activity. The present result did not show any significant changes FSH and LH whereas the progesterone level significantly changed.

The effect of LH activity was also evident in the present study but thecal luteinization was not confirmed. It has been claimed that LH conditions the steroid converting enzyme activity, Juan Zanartu; (1970) [12]. Effect of norethisterone on LH level in women also reported by Elestein (1976) [5].

No significant alterations in the ovarian histology were reported after norethisterone treatment as there were no apparent alterations in the germ cells, ovarian cortex stroma, follicular growth and thecal luteinization. All these results were probably because of FSH and LH remained unchanged, and is not enough to assure full follicular ripening or rupture, nor stimulation of activity of steroid converting dehydrogenase enzymes. Szoltys *et al*. (1994) [22] reported that LH surge was accompanied by a gradual decrease in the estradiol level, which is in accordance with our findings. It was postulated that subsequently it was progesterone that was the final product of follicular and ovarian synthesis. (Hillier, 1985) [9].

Significant decrease of serum progesterone and the regressed in corpus luteum, found in present study.

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