Effect of Progesterone Treatment on The Ovary of Albino Rat (Wistar strain). Histo-Clinical Approach

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Abstract

Progesterone is a major steroid secreted by the corpus luteum. Progesterone exists as colourless crystals or yellow-white odourless, tasteless powder. Progesterone and their effects on the specific target organs such as Ovary. It is studied as hormonal contraceptive. Progesterone is a naturally ocurring progestogen that also has antioestrogenic activity. It is available in the market as 'Uniprogesterone', and in the form of synthetic progestin. Present studies revealed the histological and clinical aspect of progesterone treated ovary.

KEYWORDS:- Uniprogesterone, antioestrogenic, contraceptive, histological, clinical.

INTRODUCTION:-

PROGESTERONE

During the last two decades, there has been a significant progress in the studies of hormonal contraceptives (Natural and Synthetic), such as Progesterone and their effects on the specific target organs. Graham et al. (1997) studied the physiological action of progesterone in target tissues such as uterus, **ovary** and mammary gland. Lee (1968) studied contraceptive and endometrial effects of medroxyprogesterone acetate, he commented that ovulation was inhibited for prolonged periods after a single injection.

Drug Chemistry

Progesterone is a major steroid secreted by the corpus luteum. In 1934, Butenandt, isolated this progestationally active substance (Butenandt and Westphal, 1934). Butenandt announced the complete synthesis of this hormone for which he and his co-workers were awarded the 1935 Nobel prize in chemistry. Progesterone exists as colourless crystals or yellow-white odourless, tasteless powder. It is prepared commercially from diosgenin or stigmasterol, which are obtained from plant sources.

Toxicology: There is limited evidence that progesterone is carcinogenic in some laboratory species (Kordon et al; 1993 and Misdorp et al; 1992), but there are no epidemiological studies in the human (WHO,1979).

MATERIALS AND METHODS

ANIMALS:

Young, healthy, sexually mature female albino rats of Wistar strain (120-150 gms body weight) with normal reproductive history were procurred from Haffkine Biofarmaceuticals. 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 .

The animals were divided into control and experimental groups, 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 treatment lasted for 24 weeks duration i.e 24 injection of i.m.injectable progesterone 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 anaesthesia. The reproductive tract was quickly excised cleared off the adhering fat blotted and weighed after which processed for the various light and biochemical studies . Simultaneously ovary was separated from the reproductive tract and processed to extract the ovarian tissue for the biochemical analysis.

LIGHT MICROSCOPIC OBSERVATION

Control Ovary:

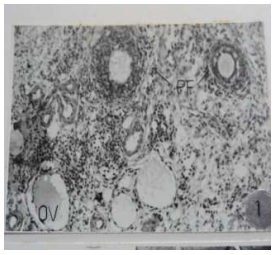


Fig. 1 control ovary section Ovum, & corpus luteum Showing the cortex, the germinal epithelium& primary follicles(PF) in various stages of developments (X- 40)

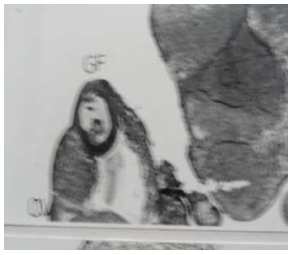


Fig. 2&3:- Showing growing follicle (GF) , (X- 40) (X- 120).

OVARY:

Histolgically 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 intestitial 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 follicli 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 folliculi 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.

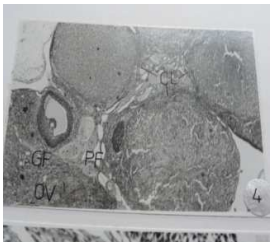
The granular cytoplasm is more distinct. The zona pellucida is increased in thickness and may be homogeneous.The theca becomes two to three layered at this stage .

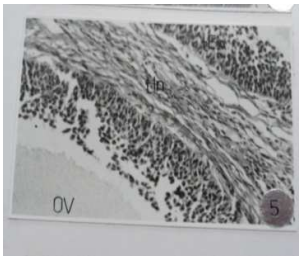
Multilaminar follicle, containes six to eight layers of follicle cells. These cells are polygonal in shape with spherical and ovoid, with a large and more or less centrally placed nuclei. The zona pellucida is increased in thickness (fig.1), the cytoplasm of the oocyte is vacuolated and the cytoplasmic 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 membrane limited lining of the antrum (fig.2,3).The liquor folliculi is an an acidophilic homogenous fluid which occupies the whole of the antrum . The oocyte surrounded by two to three layers of cumulus cells, comes to lie at one pole of the follicle, where it is broadly attached to the granulosa cells. The cells of the oophorus are more or less similar to the cells of the granulosa layer, which is four to five layers thick. The granulosa cells adjacent to the membrane propria are columnar and contain basally situated nuclei. The cytoplasm of granulosa is homogenous or granular. The oocyte contains vacuolated or reticular cytoplasm and peripheral situated nucleus, which lies adjacent to the zona pellucida . (fig.2,3).

PROGESTERONE TREATED OVARY:





Progesterone treatement for 24 weeks

Fig. 4- showing Primary follicle(PF) & growing follicle (GF) young & mature corpus luteum- X-40

Fig. 5- Theca interna and theca externa X-75.

After treatment with progesterone, the ovarian section shows mature follicle, ruptured stigma and corpus luteum. However, vesicular follicles, as well as luteinization of the internal theca in some atretic or cystic follicle is also seen (fig.4).

Histological examination of follicles, from primordial to vesicular follicles, ovarian stroma and corpus luteum revealed that these finding are similar to those observed in a normal groups of non treated rat ovary (fig.2,3). Unilaminar and bilaminar follicles are usually found but multilaminar follicles are not obsereved. Corpus luteam is similar to that of controls (fig .4). Theca interna cells of the vesicular follicles undergo hypertrophy and are vacuolated (fig. 5) .

HORMONAL ASSAY

Table No. 1 **X + SEM Value**

Sr.No.	Parameter	Control Value X1(5)	Treated Value X2 (5)
1	FSH MIU/ml	4.06 + 0.085	4.45 +1.55
2	LH MIU/M/ml	3.36 + 0.21	3.15 + 0.64
3	Oestradiol pg/ml	158.64 + 60.05	28.5 + 14.2
4	Progestrerone ng/ml	20.84 + 2.98	54.65 + 28.9
5	17(OH) Prog. Ng/ml	1.424 + 0.223	1.36 + 0.23

Results of a study in which serum FSH, LH, Progesterone, Oestradiol, 17(OH)Progesterone were serially investigated in normal female rat during a control period and after the administration of 200Ug/dl Progesterone weekly for six months indicated that progesterone did not changed LH and FSH level and those of serum progesterone and oestradiol level decreased slightly (Table No.1). Progesterone treatment did not show considerable changes in the concentration of serum 17(OH) progesterone (Table No.1) particularly in this specimen.

DISCUSSION –

In the present study after the treatment of progesterone to albino rat for 24 weeks, the ovary comprised of primary follicles, secondary follicles, Graffian follicle and corpus luteum. Theca interna was slightly thickened due to vacuolation. No significant alterations in extracellular ovarian histology were observed. There were no alterations in the germ cells, ovarian cortex stroma and follicular growth.

In the present study the changes in the ovarian structure were correlated with the hormonal essay. Increased level of progesterone could be due to the presence of corpus luteum. The thickening of theca interna may be because of the formation of vacuolation, however the relative cellular density of the theca interna layer remained unchanged.

In the present study estrogen and LH level did not showed any changes, hence full maturation of the follicle and luteinization could not possible under such circumtances.

It is possible however, that due to the sustained level of FSH and LH, Several follicullar growth may be taken place but follicle could not reach upto full maturation. Szyoltys et al. (1994), explained about slight increase in estradiol is due to fall in the

amount of androgen due to suppression which followed the inhibition of the aromatase system. This may be one of the possibility in the present study.

On the basis of the results obtained in the present experimental study we could say that the presence of corpus luteum was responsible for the increased level of progesterone. (and the corpus luteum may be from the previous ovulatory cycle because in rat the corpus luteum remains for a longer time even after the completion of ovulatory cycle (Endocrinology by Turner, 1976). Szoltys et al.(1994) reported that , the most likely ovarian structures that also responded to the LH surge were the youngest corpora lutea and their contribution could have have raised the progesterone content The present experimental evidences can not support the above result.

The second presumption was drawn that the presence of growing follicle could not interfer in the estrogen but because of reduced LH activity there was an absence of rupture, hence a definite delay of ovulation after the progesterone treatment. Lunenfeld et al; (1963) suggests that progestin may directly inhibit ovarian response to exogenous and endogenous gonadotropins, this may also good in case of rat as observed in the present study. Fotherby et al; (1977) revealed that there is no direct correlation between the plasma exposure of the steroids and gonadotropins after six month treatment in women, but in case of rats it was found that the six monthly treatment affected the gonadotropins, which clearly indicated a correlation between plasma exposure of the steroids and gonadotropins.

Present investigation proves that despite high estradiol levels in progesterone treated rats, simultaneous increase in progesterone was recorded. The normal maturation of follicle did not proceed further due to decreased level of LH, which ultimately delayed ovulation. This helped us to confirm that progesterone effects the gonadotropin output without disturbing the synthesis and secretion of steroids. Similar results have also been experienced by Valdhius et al; (1989) and in rat by Chang et al; (1984) who found that the progestogenic compounds had direct effect on ovary and they changed the biological activity of FSH and LH. These findings are quite similar to those observed in the present study.

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