ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(4): 455-459
www.allresearchjournal.com
Received: 20-02-2016
Accepted: 22-03-2016

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Effects of progesterone on the histology, histochemistry and ultrastructure of the albino rat uterus

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Abstract
Effects of progesterone on endometrium are dose dependent. The hormonal contraceptive affects the endometrium. Progestogens have an interesting and important effect upon the uterine chemistry. Changes in the activity of uterine chemistry including alkaline phosphatase studied in the mammals but rarely in rat. Rat is widely accepted as a surrogate in the study of human reproductive problems. The present study has the objective to understand the subcellular structural response to progesterone to establish a structural, functional properties concerning its contraceptive efficacy. Electron microscopy was employed to provide close intracellular scrutiny and it was not possible with light microscopy.

Keywords: Progesterone, Endometrium, Uterine chemistry, Uterine profile, Electron microscopy, Ultrastructure

Introduction
Most of the bioassays of progesterone are based upon the original observation of Turner and Allen in 1933 that the progesterone causes progestational action. Progestogen brings about changes in the endometrium of the uterus. The changes are such as to favour the implantation of a fertilized ovum and its subsequent gestation.
Naturally occurring progestogen is progesterone, which is not only important for its own endocrine effect but also because it is a precursor molecule in the synthesis of steroids in all tissues which produce them. Progesterone is secreted primarily by the corpus luteum during the luteal phase of the menstrual cycle.
Progesterone has varied actions upon the female reproductive organs and under physiological conditions, often acts synergistically with estrogen. The progesterone is capable of inhibiting the action of each other and under these conditions, are considered to act antagonistically.
Animal studies are of little relevance to humans because of differences in absorption and metabolic clearance among various species. Progesterone plays role in the regulation of reproductive cycles, but the mechanism(s) involved are poorly understood (Fotherby, 1985) [16].
The present study is designed to evaluate the possible direct influences of progesterone on the rat uterus. There is some evidence that the effects of progesterone on endometrium are dose dependent. Blood plasma concentrations are also recorded in this study. Progesterone exhibits a profound influence on both normal and abnormal endometrial tissue (Robert *et al*. 1974) [9, 28]. This investigation was designed to examine the subcellular alterations in the endometrial glands. Progesterone was administered and the various changes were studied. The objective was to study the subcellular structural response to progesterone to establish a structural, functional properties concerning its contraceptive efficacy. Electron microscopy was employed to provide close intracellular scrutiny and it was not possible with light microscopy.
The hormonal contraceptive suppresses ovulation and at the same time, affects the endometrium. The net effect of these steroids into the endometrium cannot be predicted.
The present study of the fine structure of the rat endometrium under progesterone stimulation was performed to help us acquire an insight into the fine structure of the endometrium and provide us with some knowledge regarding its efficacy as a contraceptive at the endometrial level. Progesterone had a relaxant effect on rabbit, rat and human uterus. Studies of the chemical changes in the endometrium can lead to a better understanding of its functional and structural changes. (Donald *et al.* 1956) [11].

Several authors who reported histochemical studies in the recent past, noted marked alterations in the localization of a variety of metabolic processes during the menstrual cycle (Dempsey *et al*. 1946, Atkinson *et al*. 1947, Arzac *et al.* 1948, Hall 1950 and Wislocki *et al.* 1950, Burto 1953) [10, 4, 3, 20, 30].

Progestogens have an interesting and important effect upon the uterine chemistry. Changes in the activity of uterine alkaline phosphatase during early pregnancy have been described in a number of mammalian species, including mouse (Finn and Hinchliff, 1964, Finn and McLaren 1967 and Smith, 1973) [13, 14, 27], rat (Christie, 1966 and Manning Steinetz and Giannina, 1969) [8, 23], Cow (Leiser and Wille 1970, 1972) [22], and Sheep (Hafez and White 1968; Murdoch,1970) [19, 24], but no precise physiological role has been assigned to the enzyme in any tissue or organ in which it occurs (Fernley, 1971) [12]. The enzyme in the uterus, however has been implicated in metabolic transformations concerned with the nutrition of the pre implantation embryo (Murdoch, 1970) [24] and in the induction of the decidual cell reaction (Finn and Hinchoffe, 1964, Hall 1969, Manning *et al*; 1969) [13, 21, 23].

There is also little knowledge of the nature and mechanism of action of processes responsible for the regulation of alkaline phosphatase activity in the uterus.

The present investigation is to study the changes of acid phosphatase and alkaline phosphatase activity, certain other biochemical parameters after the treatment of progesterone. However, before the rat can become widely accepted as a surrogate in the study of human reproductive problems, more information concerning its reproductive system must be obtained. This new information must not only involve the normal functioning of the system, but must also include studies on the response of the reproductive organs. The present research was undertaken in order to study the cytoarchitectural and clinical changes in reproductive organs of rat in response to a widely accepted and frequently used progestogenic contraceptive.

Although the importance of the fluids within the female reproductive tract is well recognised, there is more speculation than proof concerning their role in reproductive processes. The relatively few early studies concerned with source, composition and function of the uterine fluids have been well reviewed by Amoroso (1952) [32]. In recent years, there has been an increasing interest in defining the biochemical nature of the intraluminal environment of the bovine female reproductive tract (Nilsson 1958) [25]. The present study will support the biochemical changes occurring due to different contraceptives.

The purpose of the present study was to compare the concentrations of chemical constituents in uterus treated by hormonal contraceptives.

Variations in the neutral lipid levels in the rat uterine epithelium correlate with the reproductive state of the female (Alden 1947, Bishier and Holloway 1973) [1, 6] and depend on the periodic stimulation of the epithelial esterases by cyclic increases in the concentrations of plasma oestrogens (Boshier and Katz 1975) [6]. Such findings are highly suggestive of a specific localized role for uterine epithelial triglycerols as an energy source and a source of useful metabolites. Previous histochemical studies have been qualitatively and quantitatively nonspecific (Boshier *et al*. 1981) [5]. We undertook specific biochemical investigations to study the relations between ovarian hormone and neutral lipids particularly the triglycerols of the rat uterus after contraceptive treatment.

Materials and Methods

Animals

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 six sets 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 progesterone. Drugs were of 100% purity which is available in the market with same trade name.

Drug Chemistry

Progesterone

Progesterone is a major steroid secreted by the corpus luteum. In 1934 [7], Butenandt, isolated this progestational active substance (Butenandt and Westphal, 1934) [7]. The correct structure of progesterone was proposed by Slotta (Slotta *et al*. 1934) [31] and almost simultaneously, Butenandt announced the complete synthesis of this hormone for which he and his co-workers were awarded the 1935 Nobel prize in chemistry.

Chemistry

$\underline{21}\ \underline{30}\ \underline{2}$

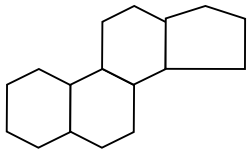
Progesterone $(C\ H\ O)$

Pregn -4 -ene - 3, 20 - dione. Molecular weight = 314.5

Solubility

In alcohol - l in 8

In water - 1 in 10,000)



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.

On the completion of the treatment period, the animals were weighed and sacrificed under light ether anaesthesia. Blood was drawn from the ventricles at the time of sacrifice. Oxalated and non-oxalated glass bulbs were used for the separation of whole blood, plasma and serum which were used for the biochemical parameters. Care was taken to avoid any hemolysis of the whole blood. The reproductive tract was quickly excised cleared off the adhering fat blotted and weighed after which processed for the various light and ultrastructural and biochemical studies. Simultaneously uterus was separated from the reproductive tract and processed to extract the uterine tissue for the biochemical analysis.

Result and Discussion

Total Uterine Profile

Table 1: X ± SEM Values

Parameter	Control values X1 (6)	Progesterone Treated values X1 (6)
Alkaline Phosphatase	60.25 ± 17.2	520.75 ± 240
Acid Phosphatase	16.3 ± 3.12	33.05 ± 11.07

(*P* >0.05 Significantly different)

Table No. 2: X ± SEM Values

Parameter	Control values X1 (6)	Progesterone Treated values X1 (6)
Sodium	156.5 ± 2.04	133.75 ± 8.87
Potassium	1.4 ± 0.315	3. 125 ± 0.98
Calcium	1.1 ± 0.115	2.8* ± 0.234
Chloride	165 ± 0.865	146.5 ± 9.97
Triglyceride	10 ± 0	12.5 ± 3.93
Cholesterol	13 ± 0.5	7* ± 2.48

(*P* > 0.05 Significantly different)

Alkaline phosphatase activity was demonstrable in the uterus of both experimental and control animals. No significant increased of alkaline phosphatase in the uterus was registered after the treatment of progesterone. Acid phosphatase activity increased under the influence of Progesterone. no significant decrease of Sodium level observed in Progesterone treated animals. No significant increase of uterine Potassium levels observed after the treatment with Progesterone. Serum Calcium levels were significantly higher in case of progesterone. The concentration of Chloride in animals treated with progesterone decreased nonsignificantly. The uterine epithelial triglycerides varied quantitatively with the reproductive state of the females. Concentration of Cholesterol was decreased significantly after Progesterone treatment.

Light and Electron Microscopy: Uterus

Light Microscopy

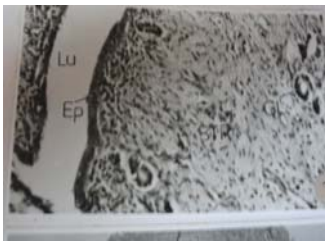


Fig a: Control Uterus- Lumen (Lu), epithelial (Ep), endometrial stroma (STR), &uterine Gland. (X-75)

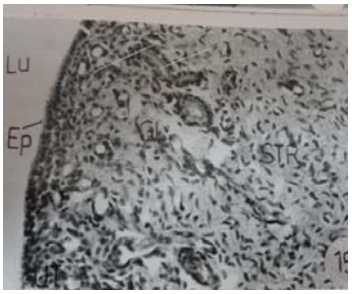


Fig b: Progesterone treated uterus- Inhibitory epithelium (Ep), atrophied uterine gland (GL), sensitized stromal cells (STR), reduced uterine lumen (Lu). (X-75)

Electron Microscopy

Ultrastructure of Uterus

Control-

Control Uterus

Fig 1: Low power electron micrograph of Endometrial epithelium of control uterus surrounding the lumen (Lu), Elongated epithelial cells (Ec), Nucleus(N) more toward the basal end, sparse chromatin network with irregular contour. (X- 2000)

Fig 2: High power electron micrograph showing scanty microvilli (MV) Mitochondria(M), Golgi apparatus(G), few profile of endoplasmic reticulum(ER), ribosomes(R), dilated inter cellular membrane. X- 10,000)

Progesterone Treated Uterus

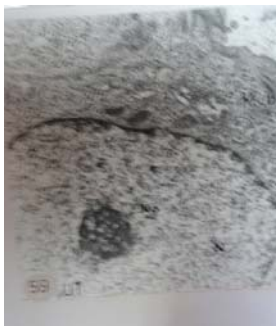


Fig 3: (X- 8000)

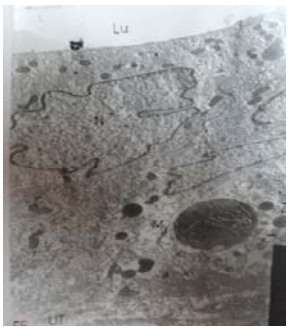


Fig 4: (X- 5000)

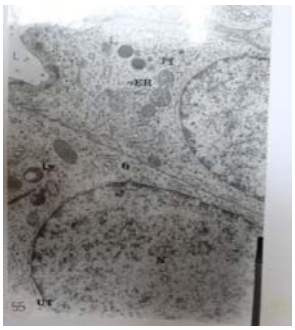


Fig 5: (8000)

Part of the epithelium after progesterone treatment under high power electron micrograph- Irregular invaginated nucleus (N), cytoplasm with zebra lipids (ZL), distended Golgi apparatus, small vesicles of rough endoplasmic reticulum (rER), spherical mitochondria (M) and bigger sized myeline figure (My)

The uterus of the control animal showed following ultrastructrural features. Fig.1 and Fig.2 showed the salient features of the supranuclear cytoplasm of the younger population of gland cells including some strands of granular endoplasmic reticulum with cisternal distension, free ribosomes, several pleomorphic (round, oval and elongated) mitochondria, prominent Golgi membrane, occasional lipid droplets and a multivesicular body. The apical surface bored microvilli, some of them had a slender shape.

The nuclei of the glandular cells of the control specimen were oval and did not exhibit a degree of irregularity in their contour (fig. 1). Nuclei were placed mostly in the middle to mid basal portion of the cell with nucleoli. Nuclear membrane was evenly approximated and regular. Nucleoli were placed peripherally (fig.1) and this inclusion assumed a central position with an ovoid, regular nucleus.

The mitochondria seen in the glandular cells of the control specimen were pleomorphic, predominantly situated in the apical portion of the cell and their cristae were infrequent, only a few extending completely across the organelle. The matrix of the mitochondria was homogeneous, some mitochondria showed loss of cristae may be because of an artifact (fig. 2).

Granular endoplasmic reticulum as described by many investigators, was common in the glandular cells of the control specimen. The rough endoplasmic reticulum appeared chiefly as randomly scattered profile of irregular vesicles and dilated short tubular structures (fig.2). Moderate number of ribosomes was present in the cytoplasm (fig. 2).

The Golgi apparatus of the glandular cells was prominently positioned apically (fig 2). The cisternae were well organised and closely approximated but occasional cisternal distension was noted (fig. 2).

Multivesicular bodies were commonly found in the apical cytoplasm and within these bodies were found variable numbers of small vesicles which were fairly uniform in size. The background matrix of the multivesicular bodies was generally of lighter density than the cytoplasm (fig. 1, 2). Lysosomes like bodies were also present.

The general cell type changed during the course of the experiment to a low cuboidal glandular cell from its original columnar shape.

The microvilli totally decreased in frequency and regularity. It was thought that the variations were attributed to absolute changes in the cytoplasmic content rather than nuclear variations. The related frequency of ciliated cells decreased under the influence of progesterone and typical cilia were not noted (fig.3, 4 and 5).

The nuclei characteristics varied after the progesterone administration with the development of nuclear membrane irregularity and invagination (fig.4).The peripherally placed nucleoli in the spherical nucleus and this inclusion at the central position with an ovoid regular nucleus (fig. 4 and 5).

After the progesterone treatment the mitochondria were similar to that of observed in control specimen and they were associated with granular endoplasmic reticulum. Mitochondria remained small, round and regular (fig. 5). The internal matrix was homogenous.

The granular endoplasmic reticulum unchanged. (fig. 5).

The Golgi apparatus was less prominent and distended, membrane vesicles were reduced in size and contained less electron dense matrix (fig.5).Multiple Golgi apparatus in one cell was a rare finding but the occurence of Golgi vesicles remained the same (fig. 5).

Lysosomes were present as there was no change as compared to the control specimen but one bigger sized myelin lysosome was observed (Fig. 5).

Notable increase of lipid inclusion after the progesterone treatment was registered. These lipid droplets were electron dense and represented as Zebra lipid (lipid droplets with bands) visualised as a structure with alternating dark and light bands (fig. 5).

The endometrium is a mirror that reflects to the most minute degree, any slightest variation in the estrogen and progesterone stimulation.

The present study demonstrates the inhibitory epithelium, glandular atrophy and reduced uterine lumen under low magnification at light microscopic level (Fig.b) whereas at ultrastructural level the microvilli totally decreased in frequency and regularity, nuclei were characterised by its irregularity and invagination and the granular endoplasmic reticulum registered an increase in frequency and a prominent Golgi apparatus also prevailed. Notable increase of lipid inclusions and 'zebra lipids' were visualised as a structure with alternating dark and light bands.

These studies have attempted to investigate the temporal pattern of progesterone uptake into various uterine cells. Gompel *et al*; (1962) [17] have shown that the hormonal alteration increases a sequence of cytodifferentiation within the glandular epithelium which is unique and specific.

In this investigation the absolute changes in the amount of nucleic acids of the tissues are not known but there was a relatively reduction of ribonucleic acid containing organelles such as polyribosomes and nucleoli after exposure to progesterone. Perhaps the intranuclear inclusion represents the dissociation of a naturally occuring inclusion. However Daniel Robert (1974) [9] conferred that the contraceptive efficacy cannot be assured on the basis of endometrial suppression at the ultrastructural level.

Prominent but not extensive Golgi membrane, unaltered mitochondria and the appearance of distended granular endoplasmic reticulum are distended and all these characterise the non secretory phase.

The biochemical parameters also support the ultrastructural findings. Progesterone stimulated glands showed demonstrable alteration in alkaline phosphatase and acid phosphatase, these findings meant that progesterone did alter the transport mechanism revealed by Flowers *et al*. (1974).

The cytoplasm of progesterone treated uterine cell was dominated by 'zebra lipids'. These types of lipids are probably the basic structure for the fat metabolism of these cells as is postulated by Nilson (1958) [25].

References

1. Alden RH. Implantation of the rat egg II. Alterations in osmiophilic epithelial lipid of the rat uterus under normal and exprimental conditIons. Anat. Rec 1947; 97:1-19.
2. Amador Gonzatez. The zoapatic VIII, ultrastructural changes in endometrium of rats. Contraception 1985; 31:5

3. Arzac JP, Blanchet E. Alkaline phosphate and glycogen in human endometrium. J clin Endocrinol. 1948; 8:315-324.
4. Atkinson WB, Engle ET. Studies on endometrial alkaline phosphatase during the human menstrual cycle and in the hormone treated monkey. Endocrinology 1947; 40:327-33.
5. Boshier DP. Triacylglycerols in the rat uterine epithelium during the oestrous cycle and early pregnancy. J Reprod Fert. 1981; 62:441-446.
6. Boshier DP. Effects of the rat blastocyst on neutral lipids and non specific esterases in the uterine luminal epithelium at the implantation area. J Reprod Fert 1976; 46:245-247.
7. Butenandt, Westphal. Zur Isolierung and charakterisierung des corpus luteum Hormones. Berl. Btsch. Chem. Ges 1934; 67:1440.
8. Christie GA. Implantation of the rat embryo: glycogen and alkaline phosphatases. J Reprod Fert 1966; 12:279-294.
9. Daniel K Robert. The ultrastructural response of human to medroxyprogesterone acetate. Am. J Obstet Gynecol. 1974; 8:811-818.
10. Dempsey Dempsey WW, Wislocki GB. J Biophus Biochem Cytol 1946, 1956; 2:743.
11. Donald, Donald G. McKay. Histochemical observations on the endometrium. (Normal edometrium). Obstet. and Gynecol 1956; 8:1.
12. Fernley HN. Mammalian alkaline phosphatases. In the enzymes, Ed. P.D. Boyer, Academic Press, New York 1971; IV:417-447.
13. Finn CA, Hinchliff JR. Reaction of the mouse uterus during implantation and deciduoma formation as demonstrated by changes in the distribution of alkaline phosphatase. J Reprod Fert. 1964; 8:331-338.
14. Finn CA, McLaren A. A Study of the early stages of implantation in mice. J Reprod Fert. 1967; 13:331-338.
15. Flower. Effects of quingestanol acetate on the histology, histochemistry and ultrastructure of the human endometrium. Am. J Obstet Gynecol, 1974.
16. Fotherby K. Oral contraceptive. Contraception 1985; 31:367-394.
17. Gompel MD. The ultrastructure of the human endometrial cell studies electron microscopy. Am. J Obstet and Gyneco. 1962; 82:8.
18. Ghadially FN. Ultrastructural pathology of the cell and matrix 3rd eds. Butterworths, 1988, I-II.
19. Hafez White, Hafez ESE, White IG. Endometrial and embryonic enzyme activities correlated with implantation in the ewe. J Reprod Fert. 1968; 16:59-67.
20. Hall JE. Alkaline phosphatase in human endometrium. Am. J Obstet Gynecol. 1950; 60:212-216.
21. Hall K. Uterine mitosis, alkaline phosphatase and adenosinc triphosphatase during development and regression of deciduomata in pseudopregnant mice. J Endocron. 1969; 44:99-100.
22. Leiser R, Wille KH. Alkaline phosphatase in the bovine endometrium and trophoblast during the early phase of implantation. Anat. Embryol 1975; 148:145-157.
23. Manning JP, Steinetz BG, Gianna T. Decidual alkaline phosphatase activity in the pregnant and pseudopregnant rat. Ann. N.Y. Acad. Sci 1969; 166:482-509.
24. Murdoch. Activity and subcellular distribution of mouse uterine alkaline phosphatase during pregnancy and pseudopregnancy. J Reprod Fert. 1970; 54:293-300.
25. Nilsson O. Ultrastructure of Mouse uterine surface epithelium under different estrogenic influence. Spayed animals and estrous animals. J Ultrastructure Research 1958; 1:375-396.
26. Pollard JW. Characterization and expression of uterine and placental alkaline phosphatases in the mouse. J Reprod Fert. 1990; 89:735-742.
27. Smith. Changes in distribution of alkaline phosphatase during early implantation and development of the mouse. Auste. J biol Sci. 1973; 26:209-217.
28. Robert Warren. An electron microscope study of the rat endometrium during delayed implantation, 1974.
29. Turner CW, Allen E. The normal and experimental development of the mammary gland of the monkey (Macaca rhesus. Anat. Rec 1933; 55:80.
30. Dempsey W.W., Burto and Wislocki, G.B.(1956). J. Biophus. Biochem Cytol.(1956); 2: 743
31. Slotta et al (1934)- Noresterate – printed drug prescription.
32. Amorso (1952)Comparative Pathology of Uterus ‘ The Uterus’