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Comparative analysis of uterine biochemistry in rat treated with world-wide popularly used contraceptives

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Abstract
Use of contraceptive is common in womens. Natural and synthetic estrogenic and progestogenic contraceptives are popularly used. Mode of intake is both in injection and oral form. Estradial valrate (E2V) Progesterone, Norethisterone, Tamoxifen and ovral. Components of estrogens and progestogens have an interesting and important effect upon the uterine chemistry. Components of estrogens and progestogens have an interesting and important effect upon the uterine chemistry. Studies of the chemical changes in the endometrium can lead to a better understanding of its functional and structural changes. Rat can become widely accepted as a surrogate in the study of human reproductive problems. 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.

Keywords: Contraceptive. Estrogen, (E2V), Progesterone, Noretisterone, Tamoxifen, Ovral, Rat, Surrogate, endometrium, uterine chemistry

1. Introduction
Estrogens and progestogens play major role as far as uterine functioning and morphology is concerned. Changes in several biochemical parameters of the immature rat uterus in response to treatment with E4 (estradiol) have been reported in previous publications (Holinka and Gurpide, 1980) [8]. In the present study the estrogenic activity of estradiol valaret and ovral was evaluated by their action on the rat uterus in relation to that of progestogen (P), Norethisterone heptanoate (Nor-Hn) and Tamoxifen compounds which differed not only in their potency but also in their tenancy time on nuclear binding sites (Anderson *et al*. 1975, Jorden *et al*. 1978, Koseki *et al*. 1977a) [1, 1].

The purpose of the present series of experiments was to extend these studies as follows- To compare the effect of Progesterone, Norethisterone (Nor-Hn) E2V(estradiol valrate), Ovral and tamoxifen on the uterine biochemistry.

1.1 Estradiol Valrate (E2V)
Estrogens have been reported to increase the chance of womb (endometrial) cancer in women who have been through menopause, especially in women who receive estrogen-only hormone therapy. Estrogens may also increase the risk of cancer of the ovary or breast. Estrogen given with another hormone (progestin) for replacement therapy Estradiol valerate is used and the amount of estrogen per dose. (Wikipedia 2016) [30].

1.2 Tamoxifen
In the late 1950s, pharmaceutical companies were actively researching a newly discovered class of anti-estrogen compounds in the hope of developing a morning-after contraceptive pill compounds never proved useful in human contraception. tamoxifen treatment alone has been shown to have anti-angiogenetic effects in animal models of cancer which appear to be, at least in par Tamoxifen did eventually receive marketing approval as a fertility treatment, but the class of compounds never proved useful in human contraception. T, independent of tamoxifen's estrogen receptor antagonist properties (Wikipedia-2016) [30].

1.3 Ovarol
This combination hormone medication is used to prevent pregnancy. It contains 2 hormones: a progestin and an estrogen. It works mainly by preventing the release of an egg (ovulation) during your menstrual cycle. It also makes vaginal fluid thicker to help prevent sperm from reaching an egg (fertilization) and changes the lining of the uterus (womb) to prevent attachment of a fertilized egg. If a fertilized egg does not attach to the uterus, it passes out of the body (Wikipedia).

1.4 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.

1.5 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.

Studies of the chemical changes in the endometrium can lead to a better understanding of its functional and structural changes. (Donald *et al*. 1956) [14]. 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) [16, 17, 18, 19,].

Components of estrogens and 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) [20, 21, 22], rat (Christie, 1966 and Manning Steinetz and Giannina, 1969) [23, 24], Cow (Leiser and Wille 1970, 1972), and Sheep (Hafez and White 1968; Murdoch,1970) [26, 27], but no precise physiological role has been assigned to the enzyme in any tissue or organ in which it occurs (Fernley, 1971).The enzyme in the uterus, however has been implicated in metabolic transformations concerned with the nutrition of the preimplantation embryo (Murdoch, 1970) [27] and in the induction of the decidual cell reaction (Finn and Hinchoffe, 1964, Hall 1969, Manning *et al*; 1969) [20, 24].

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 different estrogens and progestogens or in combination. 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.

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. 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 195a, b) [28, 29]. The present study will support the biochemical changes occurring due to different contraceptives.

2. Materials and methods

2.1 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. Animals were divided into six sets for different 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, Norethisterone (NOR-HN), Estradiol valrate, Tamaxifen and oral dose of Ovral.

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

2.2 Drug Chemistry

1. Progesterone

Progesterone is a major steroid secreted by the corpus luteum. Progesterone exists as colourless crystals or yellow-white odourless, tasteless powder. It is prepared commercially from diosgenin or stigmasterol, which are obtained from plant source.

2. Estradiol valerate

[(8R,9S,13S,14S,17S)-3-hydroxy-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[a]phenanthren-17-yl] pentanoate

3. Tamoxifen

(*Z*)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-*N*,*N*-dimethylethanamine

2.3 Ovral (combination of progesterone-estrogen)

Norgestrel-ethinyl estradiol

On the completion of the treatment period, the animals were weighed and sacrificed under light ether anaesthesia. Blood was drawn from the ventricles period to the 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.

2.4 Uterine tissue biochemistry

1. Total protein

Method: biuret method

Principle: Protein is precipitated from the Sample by Trichloroacetic acid and then

Determined by the Biuret method.

2. Total Cholesterol

Extraction Procedure: - Tissue is homogenize in saline (0.9N) centrifuged at 5000 rpm for 30 minutes. Supernatant was separated and used for cholesterol estimation.

3. Acid phosphatase (ACP) & Alkaline Phosphatase (ALP)

Method: King's Method.

Table 1: Tests were performed on Auto-analyser and the method used were –

S. No.	Investigation	Methods	Reagent
1.	Calcium	Cresolphimolein complex	Lab reagent
2.	Sodium	Ion selective electron	Lab reagent
3.	Potassium	Ion selective electron	Lab reagent
4.	Alkaline phosphatise	AMP Buffer Std. Method	Lab reagent
5.	Acid phosphatise	AMP Buffer Std. Method	Lab reagent
6.	Cholesterol	Chod Pap Method	Kit from 'E' Merk or Preccugent
7.	Triglycerides	GDP-Pap method	Kit from 'E' Merk or Preccugent

2.5 Observation

Table 2: x + sem values Alkaline phosphatase

S. no.	Drugs treated	Control values X1 (6)	Treated values X1 (6)
1	Progesterone	60.25 + 17.2	520.75 + 240
2	NOR-HN		238* + 58.1
3	Estradiol valrate		1017 + 613.34
4	Ovral		132.25* + 8.85
5	Tamaxifen		91.5 + 53.1

(P > 0.05 significantly different)

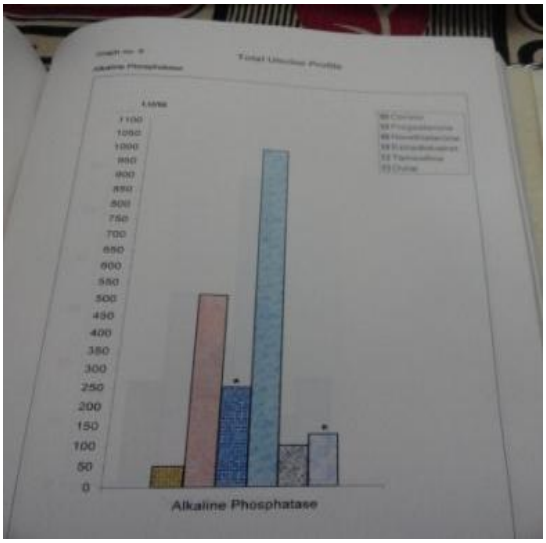

Fig 1

Table 3: Acid phosphatase x + sem Values

S. no.	Drugs treated	Control values X1 (6)	Treated values X1 (6)
1	Progesterone	16.3 + 3.12	33.05 + 11.07
2	NOR-HN		22.75 + 2.54
3	Estradiol valrate		58.25 + 20
4	Ovral		17.82 + 1.134
5	Tamaxifen		2.185* + 0.62

(P > 0.05 significantly different)

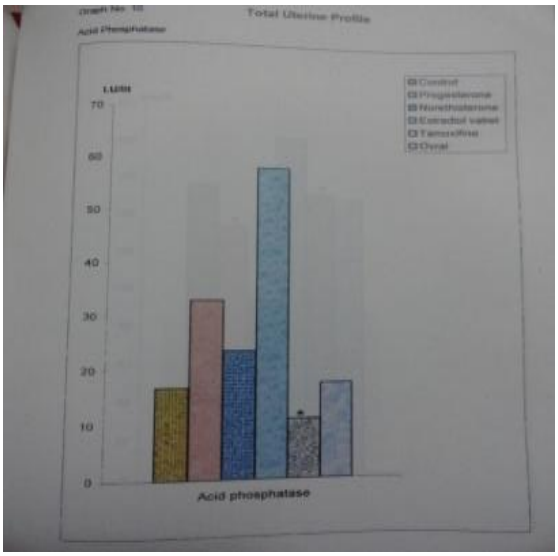

Fig 2

Table 4: Sodium x + sem Values

S. no.	Drugs treated	Control values X1 (6)	Treated values X1 (6)
1	Progesterone	156.5 + 2.04	133.75 + 8.87
2	NOR-HN		130.25* + 6.33
3	Estradiol valrate		182.75 + 18.76
4	Ovral		145.6 + 9.91
5	Tamaxifen		159.75* + 0.865

(P > 0.05 significantly different)

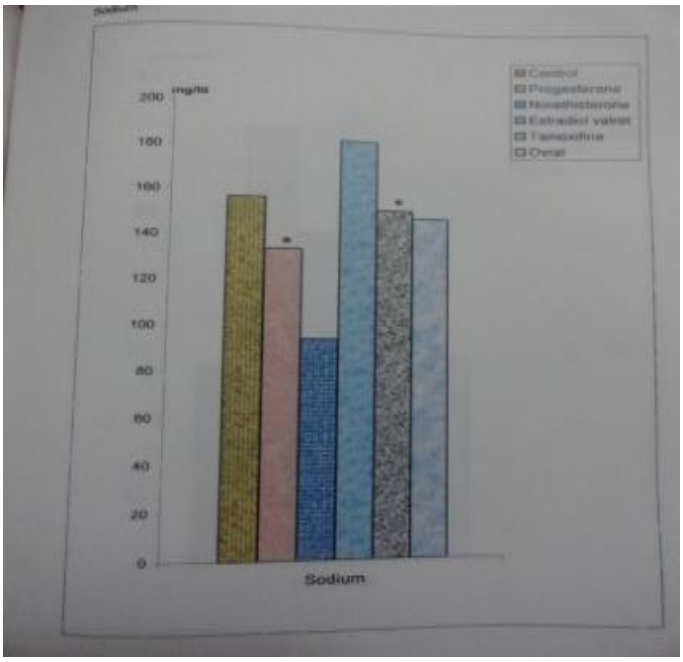

Fig 3

Table 5: Potassium x + sem Values

S. no.	Drugs treated	Control values X1 (6)	Treated values X1 (6)
1	Progesterone	1.4 + 0.315	3. 125 + 0.98
2	NOR-HN		2.3 + 0.61
3	Estradiol valrate		2.5 + 0.56
4	Ovral		1.5 + 0.145
5	Tamaxifen		0.65* + 0.16

(P > 0.05 significantly different

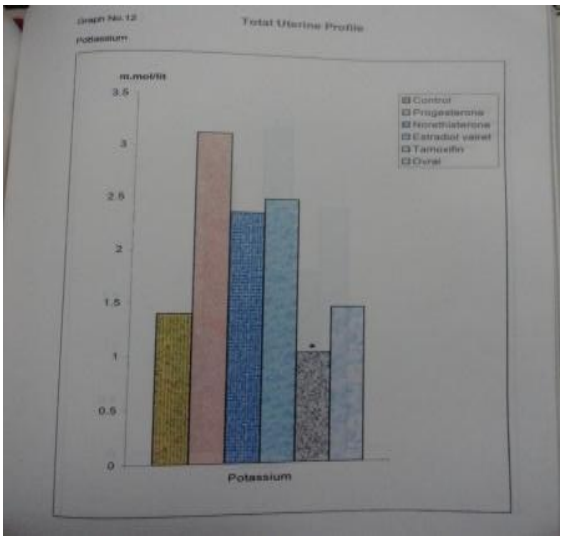

Fig 4

Table 6: Calcium x + sem Values

S. no.	Drugs treated	Control values X1 (6)	Treated values X1 (6)
1	Progesterone	1.1 + 0.115	2.8* + 0.234
2	NOR-HN		1.43 +0.31
3	Estradiol valrate		3.175* + 0.31
4	Ovral		1.75 + 0.06
5	Tamaxifen		2.375 + 0.865

(P > 0.05 significantly different)

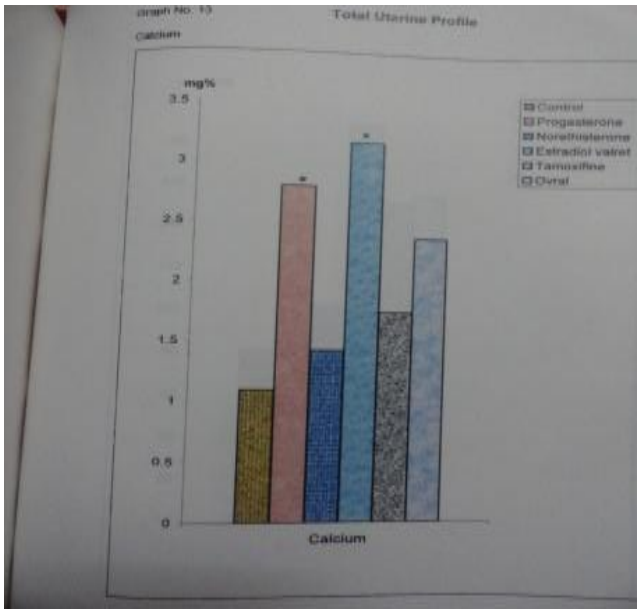

Fig 5

Table 7: CHLORIDE X + SEM Values

S. no.	Drugs treated	Control values X1 (6)	Treated values X1 (6)
1	Progesterone	165 + 0.865	146.5 + 9.97
2	NOR-HN		149.25 + 9.335
3	Estradiol valrate		190 + 20.1
4	Ovral		152 + 11.55
5	Tamaxifen		149.25 + 11.11

(P > 0.05 significantly different)

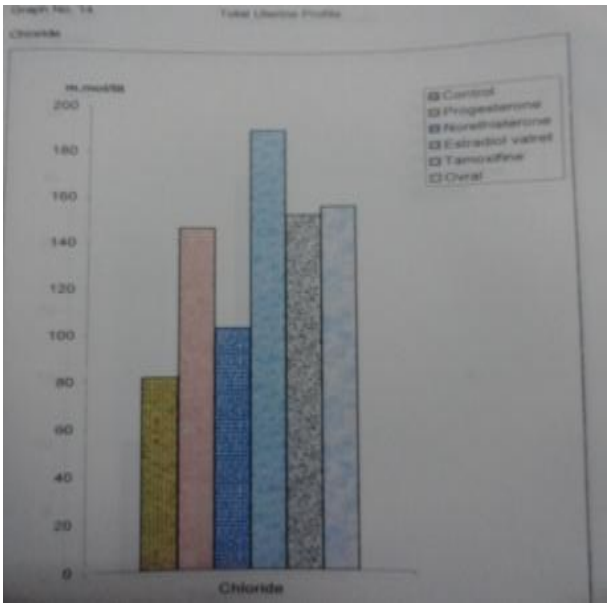

Fig 6

Table 8: Triglycerides X + SEM Values

S. no.	Drugs treated	Control values X1 (6)	Treated values X1 (6)
1	Progesterone	10 + 0	12.5 + 3.93
2	NOR-HN		8.5 + 2.3
3	Estradiol valrate		30.25 + 20.4
4	Ovral		5* + 1.62
5	Tamaxifen		7* + 1.61

(P > 0.05 significantly different

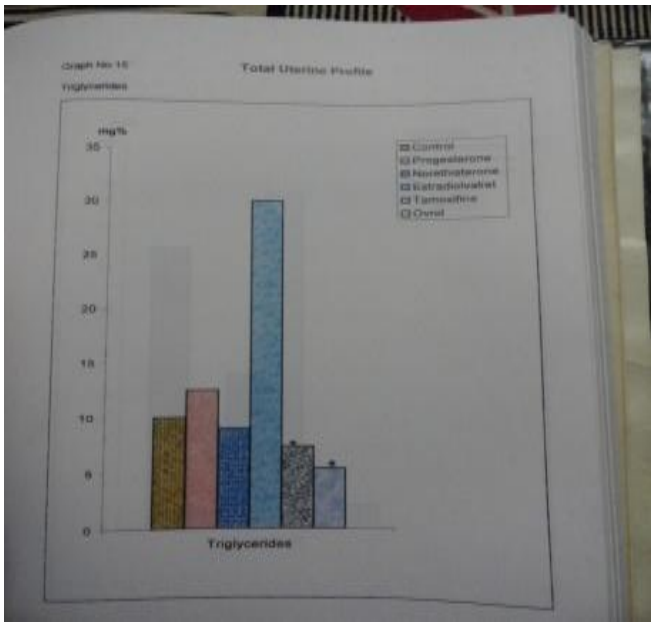

Fig 7

Table 9: Cholesterol X + SEM Values

S. no.	Drugs treated	Control values X1 (6)	Treated values X1 (6)
1	Progesterone	13 + 0.5	7* + 2.48
2	NOR-HN		7.25 + 3.04
3	Estradiol valrate		15.75 + 4.33
4	Ovral		8.75* + 1.635
5	Tamaxifen		2.5* + 0.5

(P > 0.05 significantly different)

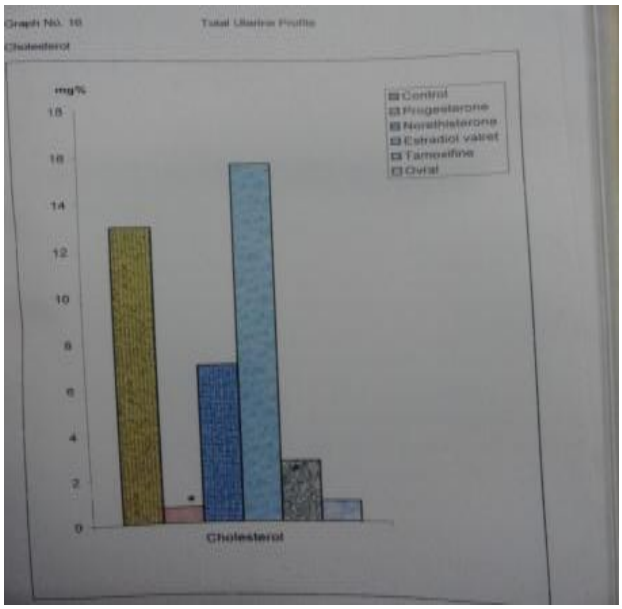

Fig 8

3. Result and discussion

3.1 Alkaline phosphatase

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, Estradiol valret, and Tamoxifen and significant increase was registered after the treatment of Ovral and Norethisterone (Table No. 2 & Fig No. 1).

3.2 Acid phosphatase

Acid phosphatase activity increased under the influence of Progesterone, E2V and Norethisteronebut it is not significant. The relative activity of acid phosphatase in the Ovral treated rat uterus is remained unchanged, whereas this activity was significantly decreased in tamoxifen treated animals (Table No. 3 & Fig No.2).

3.3 Sodium

Volumes of uterine extract collected after the different contraceptive treatments like progesterone, NOR-HN, E2V, Ovral, and tamoxifen to the rat. No considerable alterations was observed in the sodium levels of the animals treated with Ovral and Tamoxifen, whereas a significant decrease observed in Norethisterone treated animals, and no significant decrease of sodium level observed in Progesterone treated animals and Sodium level increased in estradiol valret treated animals.(Table No. 4 & Fig No.3).

3.4 Potassium

The mean potassium concentration was unchanged after the Ovral treatment. No significant increase of uterine potassium levels observed after the treatment with Progesterone, NOR-Hn and estradiol valrate and uterine potassium concentration decreased significantly after the tamoxifen treatment (Table No. 5 & Fig No. 4)

3.5 Calcium

Serum calcium levels were significantly higher in case of progesterone and estradiol valret treated animals, while no significant change was observed in norethisterone, ovral and tamoxifen treated animals (Table No. 6 & Fig No.5).

3.6 Chloride

The concentration of chloride in animals treated with norethisterone, ovral, progesterone decreased nonsignificantly while in E2V treated animals the chloride concentration peaked up but not significantly, and the level of chloride decreased significantly after the treatment of tamoxifen. (Table No. 7 & Fig No.6).

3.7 Triglycerides

The uterine epithelial triglycerides varied quantitatively with the reproductive state of the females. The levels of triglyceride present after the treatment of norethisterone, and tamoxifen were low but treatment with E2V resulted elevation but not significantly. Significantly decreased level of triglycerides found in animals treated with ovral (Table No. 8 & Fig No.7).

3.8 Cholesterol

Concentration of cholesterol was enhanced but not significantly after E2V treatment, on the other hand it was decreased significantly after Progesterone, tamoxifen and Ovral treatment and no significant decrease was observed after norethisterone treatment. (Table No. 9 & Fig No.8).

4. Discussion

The relationship of both acid and alkaline phosphatase activity to the stage of the estrous cycle compares closely with histochemical determinations of the same enzyme in the endometrium found by Kenney (1964) [7].

The uterine sodium levels after progesterone, norethisterone and ovral treatment decreased, where as sodium levels increased after E2V treatment. Tamoxifen did not alter the uterine sodium levels.

Level of potassium in tamoxifen treated rat registerd significant increase while in progesterone, Nor-HN, E2V treated rat, potassium level increased nonsignificantly. Oral administration of ovral did not alter potassium level.

Howard and Defeo (1959) [5] found that sodium level increased at the expense of potassium level in the uterine fluid. This was not the case in the present study. It is difficult to make a direct comparision between these results, however we can say that the contraceptive efficacy of hormones on these elctrolytes is independent.

Calcium concentration in uterus significantly increased after progesterone and E2V treatments, whereas in norethisterone and ovral treated animals calcium level was unchanged, tamoxifen treatment increased the uterine calcium concentration but not significantly.

The comparision of the uterus in the rat is under the control of hormones was demonstrated by the fact that the concentration of potassium, chloride, calcium, sodium alkaline phosphatase and acid phosphatase, all varied significantly with progesterone, estrogen and its compounds. The greatest variations occured due to estrogenic and progestogenic effect.

In progestogenic groups (progesterone, Norethisterone) no significant alteration was observed in uterine triglycride levels which may be due to a lack of significant effect on uterine triglyceridogenesis. The level of triglycerides in E2V treated uterus increased. Treatment with ovral significantly decreased triglyceride levels, similar results occurred in tamoxifen treated rat.

Triglycerol metabolism in the uterine epithelium reflects the reproductive state of the female which itself is under control of the ovarian hormones (Boshier *et al*; 1981) [3]. Although functional correlations of these changes in uterine triglycerol content must be conjectural (Boshier *et al*; 1981) [3]. Boshier (1976) [2], Kennedy (1977) [6] have suggested that the epithelial triglycerides would be a suitable and readily available energy and metabolites source for the use by the uterus.

In case of uterine cholesterol, significant changes are manifested by the component of estrogen and progestogen. Progesterone, norethisterone, tamoxifen and ovral administration significantly decreased the uterine cholesterol concentration, while E2V treated rat showed slight increased level of cholesterol.

Our data suggest that estrogen and progesterone alone or in combination with low potency counts for major alteration in cholesterol and triglyceride profile.

The current study is designed to give answer to, whether the activity of cholesterol is sensitive to the presence of sex

hormones. Several studies including Chico *et al*. (1994) demonstrated that the progesterone is a potent inhibitor (Lichenstein *et al*.1983 and Martinez *et al*. 1994) [11, 12]. Administration of these hormones modulates cholesterol activity.

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