

OVARIAN SENSITIVITY TO EXOGENOUS GONADOTROPHINS IN PHENOBARBITAL TREATED UNILATERALLY OVARIECTOMIZED ALBINO RATS

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Abstract : Adult cycling female rats were hemispayed and administered with 7.5 mg phenobarbital/100 gm body weight for 15 days. 5IU FSH or FSH+LH per 100 gm body weight was administered from day 13 to 15 to the hemispayed phenobarbital treated rats. Phenobarbital inhibits the ovarian compensatory hypertrophy significantly and increases the cholesterol and lipid levels in the ovary. Administration of FSH alone or in combination with LH restores the ovarian compensatory hypertrophy and decreases cholesterol and lipid levels significantly but LH alone is not much effective. These results suggest that the inhibition of ovarian growth may be due to the lack of availability of pituitary gonadotrophins in phenobarbital treated rats and these actions can be rectified by the administration of exogenous gonadotrophins which indicate that the ovary has not lost its sensitivity due to phenobarbital treatment.

Key words : phenobarbital gonadotrophins cholesterol unilateral ovariectomy

INTRODUCTION

Barbiturates block ovulation by inhibiting preovulatory surge of LH in rats and hamsters (1,2). A single injection of barbiturates is known to cause decrease in the levels of plasma FSH (1,3). The prolonged treatment of phenobarbital or barbital sodium inhibits the ovarian compensatory hypertrophy in hemispayed rats and hamsters (4). Successful attempts to counteract the barbiturate blockade of ovulation by the administration of gonadotrophins or ovarian steroids have been made (5). Therefore, it is of interest to study the ovarian response to the exogenous pituitary gonadotrophins (FSH or LH or FSH+LH) in prolonged phenobarbital treated unilaterally ovariectomized rats.

METHODS

Nulliparous rats of Wistar strain weighing 130-150 gms and 80-90 days old were unilaterally ovariectomized at estrus by removing the right ovary, under mild ether anaesthesia in semi-sterile conditions. 7.5 mg/100 gm body weight of phenobarbital (Fluka

A.G., 04710, Swiss) in 0.5 ml saline was administered subcutaneously for 15 days from the day of operation. 5IU/100 gm body weight of FSH or LH or FSH+LH was administered to these operated rats along with phenobarbital from day 13 to 15 after ovariectomy. Suitable saline or phenobarbital treated groups were served as controls. Ten rats in each group were maintained. Rats were maintained in individual cages, at room temperature ($28 \pm 2^\circ\text{C}$) with a schedule of 12 hr light and 12 hr darkness. They were fed with Central Food Technological Research Institute chow and provided water *ad libitum*. Vaginal smear was noted everyday during the period of experiment.

All the rats were autopsied by cervical dislocation on 16th day. Ovaries, uteri and adrenals were dissected out, freed from fat, weighed to the nearest mg. Ovaries were fixed in Bouin's fluid or calcium formol, sectioned in paraffin wax at 6-7 μ thickness and stained in Ehrlich's Haematoxylin - Eosin or Sudan black - B for histological or histochemical observations respectively. The ovarian cholesterol (6) and uterine RNA (7) were extracted and estimated.

RESULTS

Ovarian Hypertrophy (Table I) : Unilateral ovariectomy causes ovarian compensatory hypertrophy within 15 days. This hypertrophy of the ovary is inhibited significantly ($P < 0.001$) with 7.5 mg phenobarbital treatment for 15 days. FSH or LH treatment from 13-15 day to these unilaterally ovariectomized phenobarbital treated rats has restored the ovarian hypertrophy. The percent inhibition in the ovarian weight after phenobarbital treatment was 35.43, but it has been reduced to 24.65 or 30.75 after FSH or LH administration respectively. The ovarian hypertrophy was significantly restored ($P < 0.001$) after the administration of FSH+LH.

Ovarian Histology (Plate I) : The sham operated ovary shows fully formed corpora lutea with hypertrophied luteal cells and antral follicles (Fig. 1). The hypertrophied ovary show many developing follicles, antral follicles and freshly formed corpora lutea (Fig.

2). Phenobarbital treatment has reduced the number of growing and antral follicles (Fig. 3). Administration of FSH has increased the number of primordial follicles and antral follicles (Fig. 4). LH treatment could not induce any follicular growth (Fig. 5). But the treatment of FSH+ LH has increased all the components of ovary (Fig. 6)

Ovarian Histochemistry (Plate II) : Moderate lipid accumulation is seen in sham operated ovary (Fig. 1). Lipid accumulation is more in phenobarbital treated ovary when compared to unilaterally ovariectomized control. (Fig. 2&3). Administration of FSH+LH has decreased the lipid accumulation in the ovary of phenobarbital treated rats (Fig. 4).

Treatment with phenobarbital caused significant ($P < 0.001$) increase in the level of ovarian cholesterol as compared to control. Administration of FSH or LH or FSH+LH to phenobarbital treated rats decrease the ovarian cholesterol ($P < 0.001$).

TABLE I : Effect of gonadotrophins (FSH or LH or FSH+LH) on phenobarbital (PB) inhibited ovarian hypertrophy and cholesterol levels in unilaterally ovariectomized (UOVX) albino rats.

Group	Treatment	Ovarian weight mg/100 gm body wt. $M \pm S.E.$	Percent hypertrophy	Percent inhibition	Ovarian cholesterol $\mu\text{g}/100 \text{ mg}$ $M \pm S.E.$
A	Sham op + saline	18.76 \pm 0.56	-	-	984.7 \pm 75.5
B	UOVX + saline	28.39 \pm 0.45	51.33	-	626.4 \pm 38.7
C	UOVX + PB	18.33 \pm 1.92	-2.35	35.43	1326.8 \pm 80.7
D	UOVX+PB+FSH	21.39 \pm 0.44	19.99	24.65	1056.5 \pm 65.0
E	UOVX+PB+LH	19.66 \pm 0.62	7.26	30.75	1040.8 \pm 55.0
F	UOVX+PB+FSH+LH	28.66 \pm 1.00	56.36	-	750.0 \pm 45.0

1. Percent hypertrophy is calculated in relation to sham operated controls.
2. Percent inhibition is calculated in relation to UOVX + saline control.

Group	Ovary	Cholesterol
A:B	$P < 0.001$	$P < 0.001$
B:C	$P < 0.001$	$P < 0.001$
C:D	$P < 0.05$	$P < 0.001$
C:E	$P < 0.1$	$P < 0.001$
C:F	$P < 0.001$	$P < 0.001$

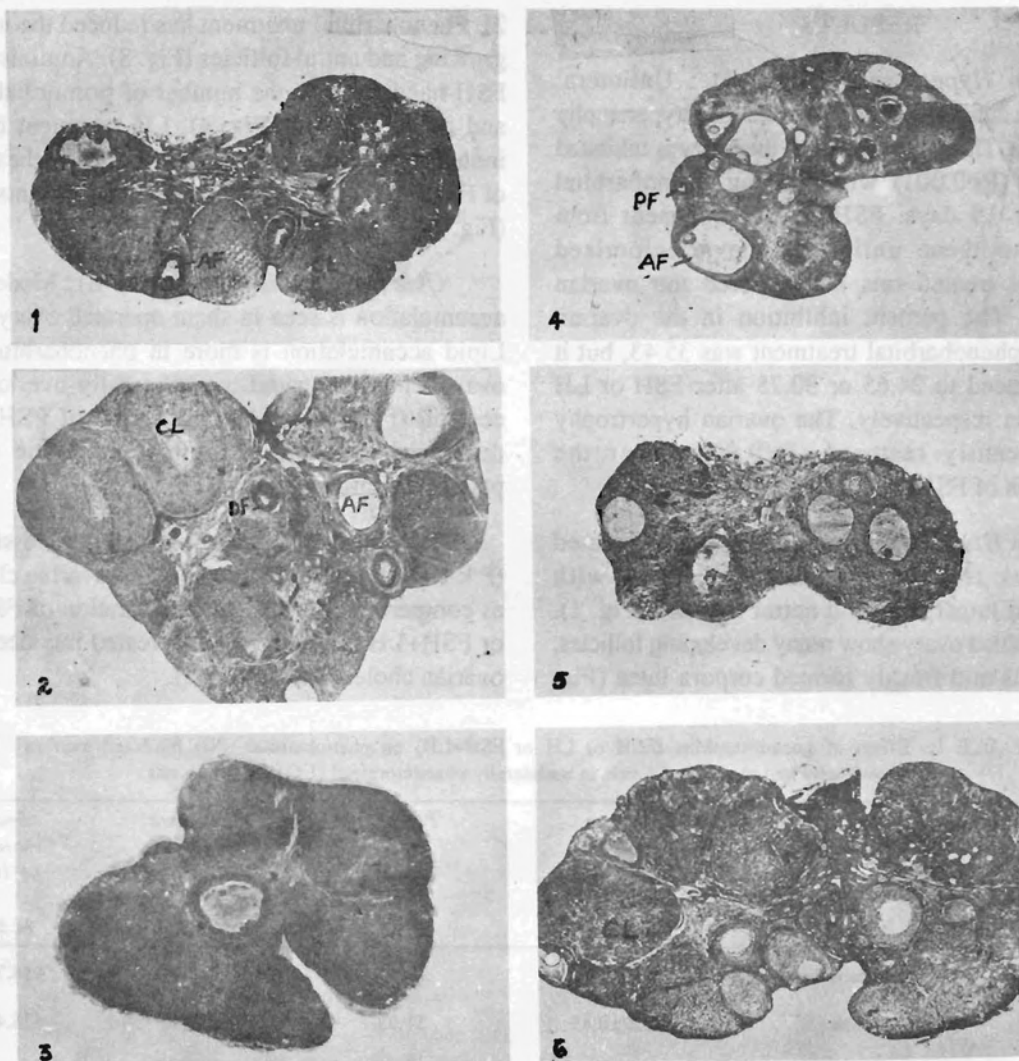


PLATE I : Cross sections of rat ovary showing histological details, stained with Ehrlich's haematoxylin - eosin x 30.

Fig.1. Sham op + saline

Fig.2. UOVX + saline

Fig.3. UOVX + Phenobarbital

Fig.4. UOVX + Phenobarbital + FSH

Fig.5. UOVX + Phenobarbital + LH

Fig.6. UOVX + Phenobarbital + FSH + LH

UOVX = Unilateral ovariectomy

AF = Antral Follicle

CL = Corpus luteum

DF = Developing Follicle

PF = Primordial Follicle

Uterine weight and its RNA content (Table II) : No significant change was observed in the weight of uterus after phenobarbital treatment. But significant change was seen in RNA content with phenobarbital and FSH+LH treatments.

Estrous cycle : Phenobarbital prolongs the diestrous period for 7-8 days with concomitant reduction in the number of cycles to 1.2. FSH or FSH+LH treatment brings the rats to estrus on the very next day in drug treated rats but LH alone was ineffective.

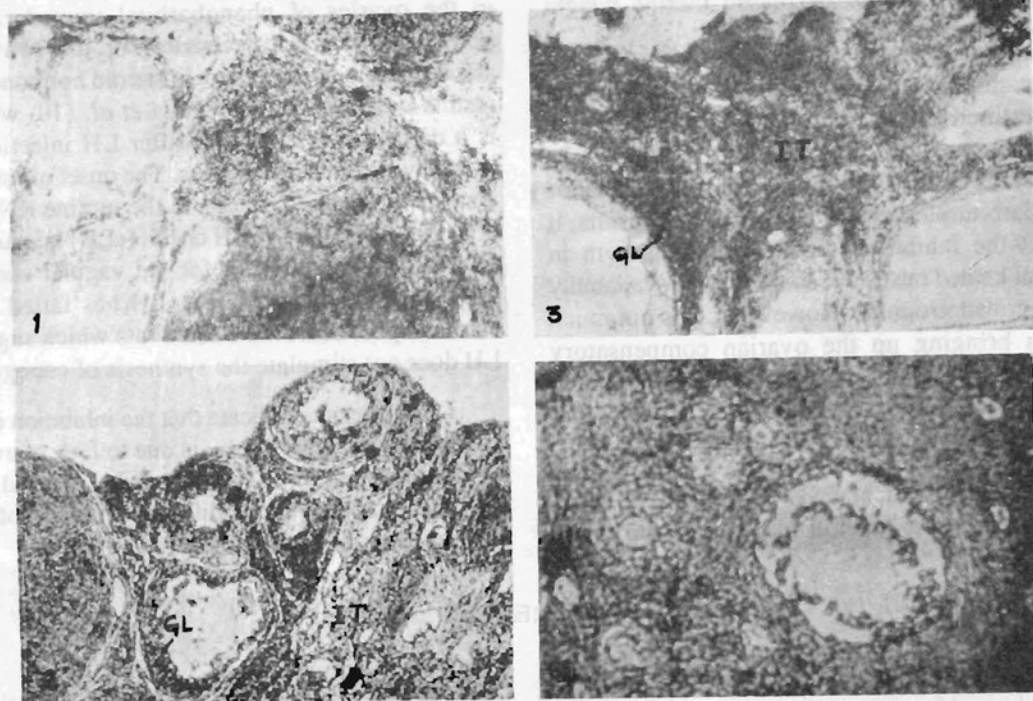


PLATE II : Cross sections of rat ovary Showing sudanophilic lipid accumulation, stained with Sudan black - B X 80

Fig.1. Sham op. + saline

Fig.2. UOVX + saline

Fig.3. UOVX + Phenobarbital

Fig.4. UOVX + FSH + LH

GL = Granulosa layer

IT + Interstitium

TABLE II : Effect of gonadotrophins (FSH or LH or FSH+LH) on uterine weight and its RNA content in phenobarbital (PB) treated unilaterally ovariectomized (UOVX) albino rats.

Group	Treatment	Uterine weight mg/100 gm body wt. \pm S.E.	Uterine RNA μ g/100 mg \pm S.E.
A	Sham op.+ saline	163.6 \pm 18.0	230.0 \pm 10.0
B	UOVX+ saline	172.5 \pm 28.1	262.8 \pm 12.0
C	UOVX+ PB	143.1 \pm 32.3	205.0 \pm 18.5*
D	UOVX+PB+FSH	175.6 \pm 10.9	255.0 \pm 18.0
E	UOVX+PB+LH	163.3 \pm 15.6	230.0 \pm 17.0
F	UOVX+PB+FSH+LH	184.4 \pm 10.7	265.5 \pm 9.0*

*P < 0.001 compared to control.

DISCUSSION

Several investigations indicate that the administration of barbiturates prior to the critical period on proestrus day block ovulation by inhibiting preovulatory LH surge from the pituitary in rats (2). Studies also reveal that the plasma level of FSH is lowered after barbiturate injections (3,8). In the present study the treatment of phenobarbital continuously for 15 days inhibits the ovarian compensatory hypertrophy, which may be due to the continuous inhibition in the release of gonadotrophins from the pituitary. The high level of cholesterol and lipid accumulation observed in the drug treated ovary indicates the inhibition of steroid hormone synthesis, as the process of steroidogenesis is gonadotrophin dependent. This altered steroid hormone production in the ovary is also evidenced

by prolonged diestrus and decreased uterine weight and its RNA content.

Administration of FSH alone or in combination with LH restored all the activities of the ovary in phenobarbital treated rats. This indicates that the ovary has not lost its sensitivity to gonadotrophins after prolonged barbiturate treatment. From these results, it is clear that the inhibition in the ovarian growth in phenobarbital treated rats is due to the lack of availability of pituitary gonadotrophins. However, LH is not much effective in bringing up the ovarian compensatory hypertrophy in drug treated animals as LH is responsible for causing ovulation and not follicular development (9).

The administration of FSH or LH or FSH+LH causes reduction in the level of cholesterol and lipids

in the ovaries of phenobarbital treated rats as the gonadotrophins are necessary for the conversion of steroid hormone precursors to steroid hormones. Similar results were obtained by Taya *et al.* (10) where there is a depletion in the lipids after LH injections in the hypophysectomized hamsters. The onset of estrus on the very next day and increase in the uterine RNA content after gonadotrophins (FSH or FSH+LH) injections reveal that estrogens necessary for the vaginal cornification have been synthesized. But, LH has failed to induce estrus in phenobarbital treated rats which suggests that LH does not stimulate the synthesis of estrogen.

These results indicate that the inhibition of ovarian compensatory hypertrophy is due to lack of availability of gonadotrophins in phenobarbital treated rats and phenobarbital does not modify the sensitivity of receptors for gonadotrophins in the ovary.

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