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Effect of estradiol on corpus luteum function in pregnant hamster

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Abstract

It was found that parturition in the hamster is associated with low circulating and luteal progesterone, low tissue-bound LH in corpus luteum (CL), high esterified cholesterol in CL, high circulating LH and peak levels of estrogen in serum, CL and the non-luteal ovarian tissue. The high concentrations of estrogen suggested a role for this steroid hormone in the regulation of corpus luteum function. Hence the effect of estradiol on the *in vivo* and in *vitro* functionality of corpus luteum was studied. It was found that administration of estradiol to pregnant hamsters resulted in a drastic reduction of progesterone levels in the serum. A direct inhibitory effect of estradiol on the progesterone production by the corpus luteum *in vitro* was observed. This inhibition of progesterone production occurred between 30 and 45. LH responsiveness of corpus luteum diminished considerably in presence of estradiol.

Keywords : Corpus luteum, estradiol, hamster.

1. Introduction

Greenstein *et al*[1] implicated estrogens in luteal control. They reported that daily injections of estrogen during the estrous cycle caused early regression of bovine corpus luteum. This luteolytic effect of estrogen was subsequently demonstrated in other species as well[2–5]. Estrogen was found to be luteolytic in the ewe when administered only in the latter half of the cycle. Moreover prior treatment with hCG diminished the ability of estrogen to induce luteolysis, suggesting that the effect of estrogen may be reversible[6]. Administration of estradiol benzoate to rhesus monkeys resulted in lowered plasma progesterone levels without at the same time interfering with serum

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LH concentrations[7], or the ability of the corpus luteum bearing ovary to secrete more estrogen than the other ovary during the late luteal phase[8], pointing to the possibility of a direct action of estrogen on the corpus luteum. However, a detailed analysis of the effect of estradiol on the luteal functionality in the hamster has not been studied so far. The present study is an attempt to understand the action of estradiol on corpus luteum function in the pregnant hamster.

2. Materials and methods

2.1. *Hamsters*

Colony-bred adult female golden hamsters (*Mesocricetus auratus*) were caged with adult males and checked daily for presence of vaginal sperm. Day 1 of pregnancy was designated as that day on which the vaginal smear was sperm positive.

Animals were always sacrificed on the prescribed day between 10 a.m and 12 noon. Blood collected from the abdominal and inferior vena cava was centrifuged and the serum was stored frozen until assayed. Ovaries were dissected, rinsed in ice-cold saline, and freed of adhering fat. The corpora lutea were separated from the non-luteal tissue, and both were weighed to the nearest 0·05 mg and frozen until analysis.

2.2. *Assay of free and esterified cholesterol*

Extraction, separation of free and esterified cholesterol and estimation of cholesterol were performed as described earlier[9]. Cholesteryl ester was estimated directly without saponification. Therefore, the values for cholesteryl ester are reported as cholesterol equivalents.

2.3. *Radioimmunoassay (RIA) of hormones*

Progesterone and estrogen were assayed according to methods previously described[9]. Progesterone was estimated using 1,2,6,7, –^{3}H – progesterone and antiserum (1 : 20,000 final dilution) produced in rabbits immunised with progesterone-11-succinyl-BSA (gift of Dr. H. R. Behrman). Cross reaction of the antiserum with 20 α–hydroxy progesterone and 17 α–hydroxy progesterone was 7% and 2% respectively. Sensitivity of the assay was 25 pg.

Estrogen was assayed using 2,4,6,7–^{3}H estradiol and an antiserum (1 : 15,000 final dilution) raised in rabbits against estradiol–17B–succinyl–BSA (gift of Dr. B. V. Caldwell). This antiserum cross reacts with estradiol and estrone, and the estrogen values obtained, as such, are expressed as total estradiol equivalents. Sensitivity of the assay was 50 pg.

2.4. *LH*

LH in serum and tissues was estimated according to the methods of Moudgal *et al*[10] as described below. LH was estimated using the components of the NIAMDD rat LHRIA kit. The only change here was that the rat LH a/s was replaced by an a/s to oLH, which could bind 30-40% of ^{125}I–rLH at an initial dilution of 1 : 40,000, the values are expressed as ng/mg tissue in terms of NIAMDD rat LH-RP standards. Serum LH was similarly assayed in duplicates and the values expressed as ng/ml serum. Sensitivity of the assay was 10 ng.

2.5. *Binding of ^{125}I-hCG to luteal tissue*

The binding assay of luteal homogenates to labelled hCG was conducted in Tris-HCl buffer, pH 7·4 (containing 1 mM $MgCl_2$ and 0·1% BSA) at 37° C for 1 h as described eariler[11].

2.6. *Incubation of isolated corpora lutea* in vitro

Corpora lutea were incubated in minimal essential medium (MEM) at pH 7·2 and 37° C as described earlier[12]. At the end of incubation, the contents of the incubation flasks were snap-frozen in liquid N_2 and stored until analysis.

3. Results

3.1. *Hormone and cholesterol levels in serum, CL and NL ovarian tissue in the pregnant hamster*

Serum progesterone increased till day 14, falling rapidly after parturition, whereas progesterone levels remained almost constant in the corpus luteum during pregnancy; post-parturition levels were, however, reduced (Table I). Serum estrogen levels, on the other hand, increased dramatically by day 15 of pregnancy. A gradual rise in estrogen was also observed in the luteal as well as the non-luteal compartment of the ovary (Table II). Free cholesterol levels in the corpus luteum markedly decreased on day 12 of pregnancy, whereas esterified cholesterol increased on day 16 compared to day 8 concentrations. In the non-luteal compartment of the ovary, free and esterified cholesterol contents were highest on day 14 of pregnancy compared to days, 8, 12 and 16 (Tables III and IV).

A steady fall in the serum LH values was observed till day 14 of pregnancy, followed by a rapid rise just after parturition. Highest concentration of tissue-bound LH was seen in the corpus luteum on day 14, whereas in the non-luteal tissue this occurred on day 12. However, the ability of both the compartments of the ovary to sequester highest amounts of LH from the circulation appears to occur on day 14 (Table V).

Table I

Progesterone levels during pregnancy of hamster

Day of Pregnancy	Serum P ng/ml*	Luteal p ng/mg tissue*
8 (10)	6·9 ± 0·1	23·9 ± 5·5
12 (9)	12·0 ± 1·4	25·8 ± 8·1
14 (10)	15·1 ± 2·2	26·5 ± 2·8
16 (10)	1·1 ± 0·3	7·6 ± 0·2

* Mean ± S.D

Numbers in parenthesis indicate the number of animals used for each experiment.

Serum values

Day 8 *vs* 12 $p < 0·001$
12 *vs* 14 $p < 0·001$
14 *vs* 16 $p < 0·001$
8 *vs* 16 $p < 0·001$
12 *vs* 16 $p < 0·001$
8 *vs* 14 $p < 0·001$

Luteal progesterone

Day 8 *vs* 16 $p < 0·001$

Table II

Estrogen levels during pregnancy of hamster

Day of pregnancy	Serum (pg/ml)*	CL pg/mg tissue*	NL pg/mg tissue*
8 (6)	284 ± 18	155 ± 21	174 ± 32
12 (6)	378 ± 25	184 ± 14	238 ± 36
15 (6)	762 ± 47	228 ± 40	370 ± 36

* Mean ± S.D CL = corpus luteum NL = non-luteal tissue.
Numbers in parenthesis indicate the number of animals used for each experiment.

Serum

Day 8 *vs* 15 — $p < 0·002$
12 *vs* 15 — $p < 0·002$

CL

Day 8 *vs* 15 — $p < 0·05$
12 *vs* 15 — $p < 0·05$

NL

Day 8 *vs* 15 — $p < 0·002$
12 *vs* 15 — $p < 0·05$

Table III

Concentration of luteal cholesterol during pregnancy of hamster

Day of pregnancy	Cholesterol μg/mg tissue*			
	Free	Ester	Total	E : F
8 (7)	3·0 ± 0·2	1·9 ± 0·6	4·9	0·6
12 (9)	1·7 ± 0·3	2·9 ± 0·1	4·6	1·7
14 (6)	2·2 ± 0·2	2·8 ± 0·7	5·0	1·3
16 (8)	3·3 ± 0·4	3·0 ± 0·1	6·3	0·9

* Mean ± S.D of three determinations.
Numbers in parenthesis denote the number of animals per group. Each experiment was repeated at least twice.

Ester cholesterol
Day 8 *vs* Days 12, 14, 16 $p < 0·05$

Free cholesterol
Day 8 *vs* Day 12 $p < 0·05$

Table IV

Non-luteal ovarian cholesterol content during pregnancy of hamster

Day of pregnancy	Cholesterol μg/mg tissue*			
	Free	Ester	Total	E : F
8 (7)	1·4 ± 0·1	1·8 ± 0·4	3·2	1·3
12 (9)	0·9 ± 0·2	1·0 ± 0·1	1·9	1·1
14 (6)	2·2 ± 0·4	3·4 ± 0·4	5·6	1·5
16 (8)	1·2 ± 0·3	2·4 ± 0·3	3·6	2·0

* Mean ± S.D of three determinations.
Numbers in parenthesis denote the number of animals used per group. Each experiment was repeated twice.

Free cholesterol
Day 8 *vs* Day 12 and 14 $p < 0·05$

Ester cholesterol
Day 12 *vs* Days 14 and 16 $p < 0·002$

.2. *Effect of estradiol administration* in vivo *on the functionality of the corpus luteum*

Administration of estradiol (110 μg/animal) on day 1, 2 or 3 of pregnancy to the animals prevented implantation, whereas estradiol was without any effect on the course of gestation when injected to animals of either day 4 or 7 of pregnancy (Table VI). When

Table V

LH profile of pregnant hamster

Day of pregnancy	Serum ng/ml	Luteal* ng/mg	Luteal LH expressed as % of serum LH	Non-luteal* ng/mg	Non-luteal LH expressed as % of serum LH
8 (7)	89·8 ± 8·0	5·6 ± 0·8	6·2	3·1 ± 0·6	3·4
12 (10)	69·3 ± 12·2	2·9 ± 0·8	4·2	5·1 ± 0·3	7·3
14 (12)	34·2 ± 8·4	6·3 ± 0·9	18·4	3·7 ± 1·0	10·8
16 (10)	94·6 ± 13·9	3·4 ± 0·2	3·6	2·8 ± 0·5	3·0

* Mean ± S.D.

Numbers in parenthesis denote the number of animals used per group. Each experiment was repeated at least twice.

		Serum	*Luteal*	*Non-luteal*
Day	8 *vs* 12	$p < 0·001$	$p < 0·002$	$p < 0·001$
	12 *vs* 14	$p < 0·001$	$p < 0·002$	$p < 0·002$
	14 *vs* 16	$p < 0·001$	$p < 0·001$	Not significant

estradiol was administered to day 8 or 12 pregnant animals serum progesterone levels were significantly reduced, whereas in the case of day 15 animals, estradiol did not seem to have any effect (Table VII). An examination of progesterone content of luteal and non-luteal tissues of pregnant animals treated with estradiol, revealed no significant change, as compared to controls (data not presented), unlike the picture seen with serum progesterone levels. In addition, serum LH levels measured on different days of pregnancy, after estradiol treatment did not show any statistically significant difference as compared to controls (data not shown). There was no difference in the ability of the luteal tissue, obtained from estradiol treated animals, to bind to ^{125}I-hCG, as compared to controls (Table VIII).

3.3. *Effect of estradiol* in vitro *on the functionality of the corpus luteum*

The observation that estradiol administration to pregnant animals of day 8 resulted in decreased progesterone levels in serum, without at the same time affecting the serum LH levels or the ability of estradiol–treated luteal tissue to bind labelled hCG indicated the possibility of estradiol acting directly on the corpus luteum. Experiments were, therefore, conducted *in vitro* to examine the effect of estradiol on luteal function.

It appeared that day 8 corpora lutea were more sensitive to exogenous steroids, as compared to day 12 corpora lutea. Estradiol, at concentration of 0·5 μg/ml inhibited

Table VI

Effect of estradiol-17β on the course of gestation

Day of pregnancy on which estradiol was administered	Day of pregnancy on which animals were autopsied	Pregnant/ Not pregnant
1 (12)	8	Not Pregnant
2 (7)	8	Not Pregnant
3 (6)	8	Not Pregnant
4 (13)	8	Pregnant
7 (9)	14	Pregnant

Estradiol (100 μg/animal) prepared in 0·1 ml of propylene glycol was injected i.p. Control animals received 0·1 ml of propylene glycol.

Numbers in parenthesis denote the number of animals used per group. Corresponding controls were maintained for each experiment.

Table VII

Effect of estradiol-17β serum progesterone levels

Day of pregnancy*	Progesterone ng/ml[@] Control	Estradiol
8	4·5 ± 0·4 (4)	2·7 ± 0·8 (4)
12	16·2 ± 3·0 (6)	7·5 ± 1·0 (4)
15	4·7 ± 1·6 (4)	5·2 ± 1·2 (4)

Mean ± S.D.

*Estradiol (100 μg/animal) was administered on the indicated day of pregnancy and animals were autopsied after 4 hr.

Day 8—control *vs* estradiol $p < 0·05$
12—control *vs* estradiol $p < 0·002$
15—control *vs* estradiol Not significant

Numbers in parenthesis denote the number of animals per group.

Table VIII

Effect of estradiol-17β on ^{125}hCG binding to luteal tissue

Day of pregnancy*	opm/mg tissue[a] Control	Estradiol
8	8108 ± 1129 (6)	6683 ± 1232 (5)
12	6786 ± 1615 (7)	6935 ± 1714 (6)
15	3612 ± 1001 (5)	3437 ± 572 (7)

Mean ± S.D.[a]

*Estradiol (100 μg/animal) was administered on the indicated day of pregnancy and animals sacrificed after 4h, luteal tissue was collected and the binding assay was performed.

Numbers in parenthesis indicate the number of animals used per group.
Results are not statistically different.

progesterone secretion by 44% and 21% by day 8 (Fig. 1) and 12 (Fig. 2) corpora lutea, respectively. On the other hand, even at a concentration of 5 μg/ml, estradiol was found to be not effective in inhibiting progesterone secretion by the day 15 corpora lutea (Fig. 3). Testosterone and DHT, used to check the specificity of estradiol action appeared to be less potent compared to estradiol.

Day 8 corpora lutea, being most sensitive to estradiol, were used in all further experiments.

A time course study revealed that inhibition of progesterone secretion by estradiol was evident by about 30′, although a clear cut effect was observed only at 60′ (Fig. 4). The responsiveness of the corpora lutea to exogenous LH *in vitro* was inhibited by estradiol as evidenced by a reduction in the amount of progesterone secreted (Table IX).

Table IX

Effect of estradiol on the responsiveness of corpora lutea to LH

	Progesterene ng/mg tissue/2h*
Control	44·7 ± 2·3
LH	99·1 ± 19·4
Estradiol	25·9 ± 3·3
LH + Estradiol	59·1 ± 12·8

* Mean ± S.D. of triplicate determinations.
LH (Sairam) 5 μg/ml
Estradiol 17-β 1 μg/ml
Corpora lutea were incubated in 1 ml of MEM buffer at pH 7·2, 37° for 2h. Progesterone was estimated in medium by RIA.

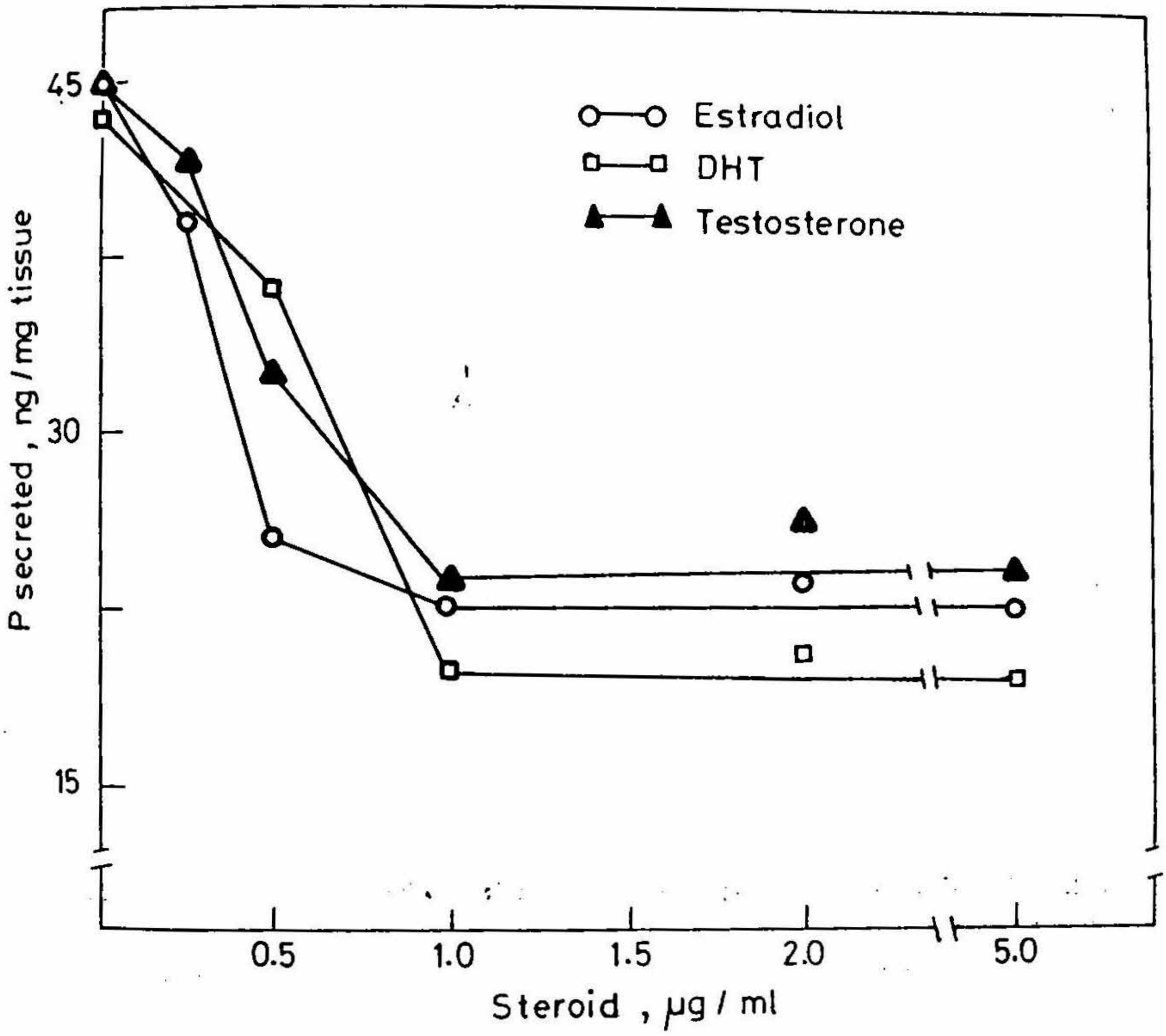

FIG. 1. Effect of steroids on progesterone secretion *in vitro* by corpora lutea of day 8 pregnant hamsters.

The steroids were dissolved in propylene glycol and used in volumes of 5–10 µl (propylene glycol was found not to affect progesterone secretion). Corpora lutea (5–10 mg) were incubated in 1 ml MEM buffer at pH 7·2, 37° for 2h, Progesterone was estimated in the medium by RIA. Each point represents mean of duplicate determinations. Each experiment was performed twice at least.

4. Discussion

4.1. *Functionlitay of the hamster corpus luteum during pregnancy*

In the corpus luteum, until day 15 of pregnancy the ratio of esterified : free cholesterol was approximately 1, according to the observations made by Chatterjee and Greenwald[15],

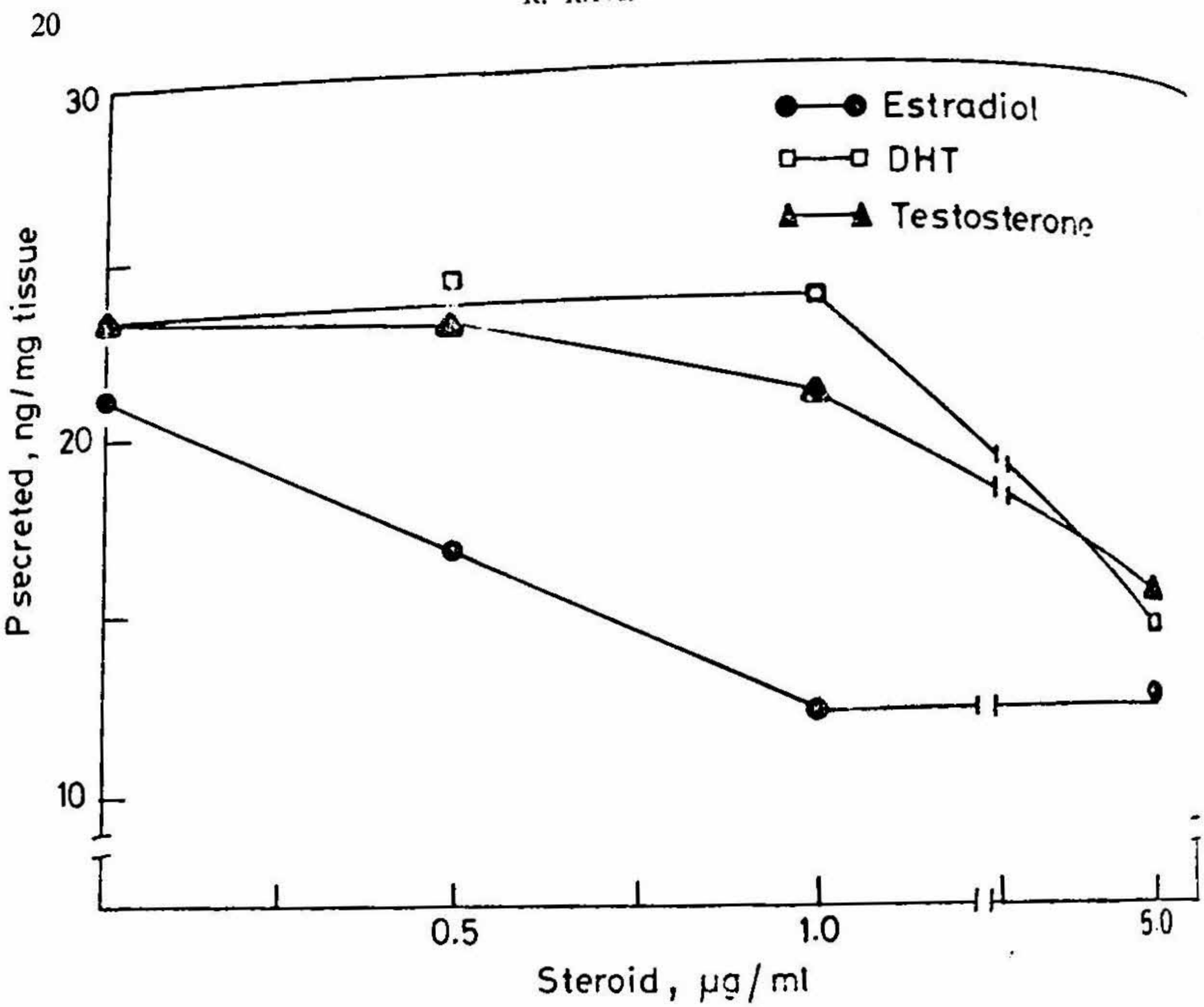

FIG. 2. Effect of steroids on progesterone secretion *in vitro* by corpora lutea of day 12 pregnant hamsters.

Steroids, dissolved in propylene glycol, were used in volumes of 5–10 µl. Corpora lutea were incubated in 1 ml MEM buffer at pH 7·2, 37° for 2h. Progesterone was estimated in the medium by RIA. Each point represents mean of duplicate determinations.

This, however, is contradictory to an earlier report[16] that the ratio on day 12 was 0·33. On the other hand, the ratio of 1·7 was obtained for day 12 corpus luteum in this study. Chatterjee and Greenwald[15] reported high levels of esterified cholesterol on day 16 (esterified : free is 2·5), whereas according to the present study as well as an earlier report[17], the E : F ratio on day 16 varied from 0·9 to 1·9. As regards the non-luteal tissue, the E : F ratio observed in this study (1·1) is similar to values reported earlier[6]. An appreciable decrease in the total cholesterol (free + esterified) on day 12 suggests a possibility of the usage of these precursors towards the production of estrogen. Considering the large amount of steroidogenesis taking place during pregnancy, one would expect a reduction in the luteal cholesterol ester levels but the data suggests no correlation whatsoever between progesterone production and levels of the steroid precursors.

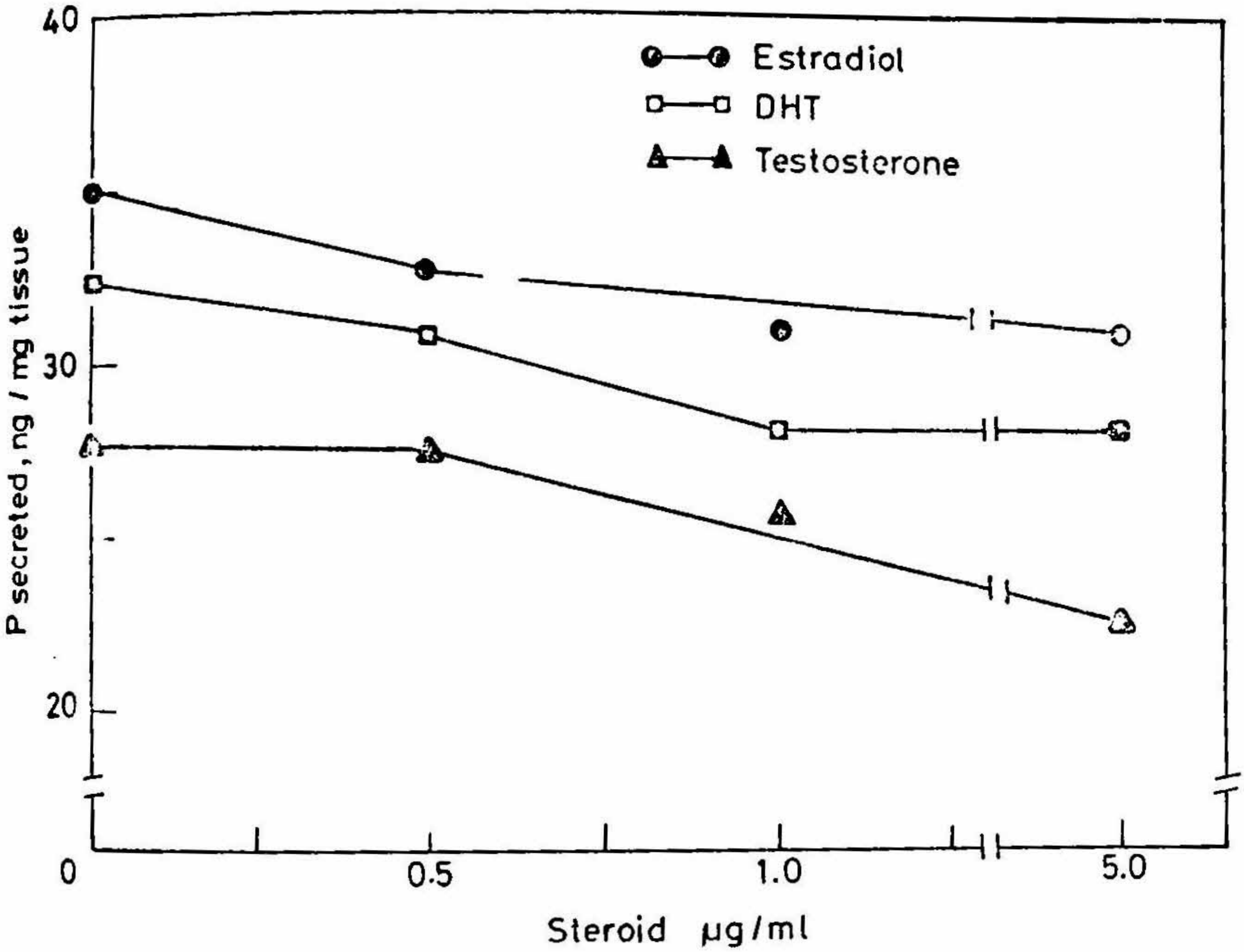

FIG. 3. Effect of steroids on progesterone secretion *in vitro* by corpora lutea of day 15 pregnant hamsters.

Steroids, dissolved in propylene glycol, were used in volume of 5–10 µl. Corpora lutea were incubated in 1 ml MEM buffer at pH 7·2, 37° for 2h. Progesterone was estimated in the medium by RIA. Each point represents mean of duplicate determinations.

Though the actual levels of tissue-bound LH in the non-luteal compartment of the ovary were maximal on day 12, the ability of day 14 non-luteal tissue to sequester circulating LH was the highest (10% on day 14 *vs* 3–7% on other days). The corpora lutea of day 15, which are on the verge of luteolysis, exhibited a remarkable incapacity to bind 125_I hCG in contrast to day 8 corpora lutea, which represent functionally active state (Table VIII). Thus, a local deprivation of LH seems to occur on day 16, post-parturition, and this might represent an important factor in the onset of luteolysis. It appears that day 14 is a pivotal point in luteal function. The luteal and serum progesterone are maximal on that day and the LH concentration of the corpus luteum also appears to be the highest on day 14; also the luteal compartment has acquired an ability to sequester a greater concentration of serum LH than that on the other days (18% on day 14 *vs* 4–6% on other days). This could be due to an

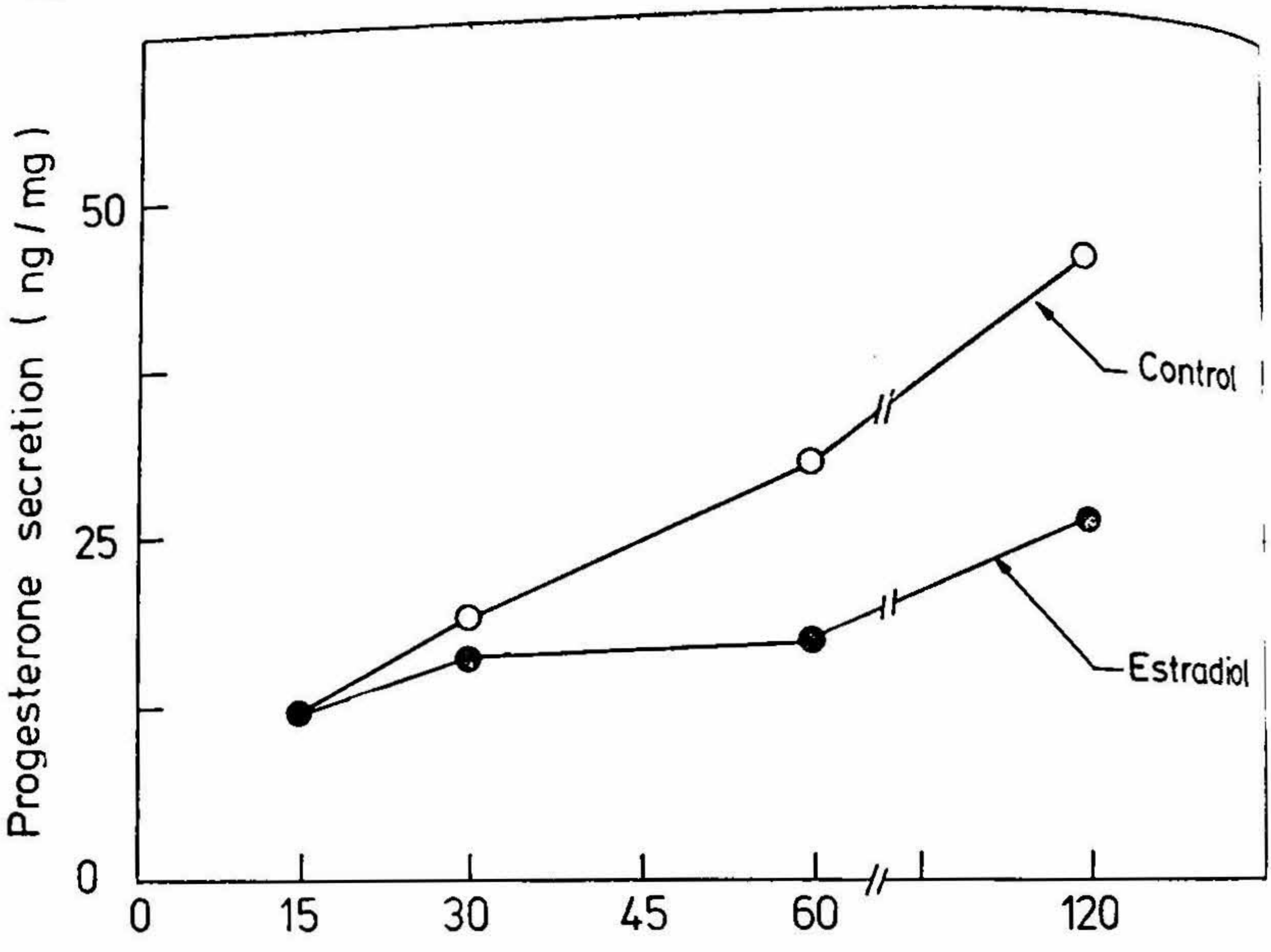

FIG. 4. Time course of estradiol inhibition of progesterone secretion *in vitro* by corpora lutea of day 8 pregnant hamsters.

Corpora lutea were incubated in 1 ml MEM buffer, pH 7·2, 37°. At particular time points the flasks containing the corpora lutea were snap-frozen using liquid N_2 and stored until further analysis. Progesterone was estimated in the medium by RIA. Each point represents mean of two determinations. This experiment was repeated twice. Estradiol was used at a concentration of 1 μg/ml.

increase in the gross number of luteal LH receptors or an increase in affinity. The question arises whether saturation of receptors with LH by itself is a signal for luteolysis.

The maximum progesterone levels in the serum observed on day 14 of pregnancy correlate quite well with luteal LH on that day, which is also maximal. The significant drop in serum LH at this time point could be due to the feedback effect of progesterone on the pituitary. The progesterone profile reported here is similiar to the pattern obtained by earlier workers[14]. The luteal progesterone remained more or less constant during the gestation, the declining, however, following parturition. The steady increase in estrogen in all the three components *viz.*, serum, luteal and non-luteal tissues as the gestation period is nearing its end, points the possibility of this steroid participating in the luteal regulation,

4.2. *Effect of estradiol* in vivo *on the luteal function*

Though estradiol could prevent implantation, it could not interrupt the pregnancy once the implantation process has occurred, as evidenced by lack of any effect on the course of gestation, when administered to day 4 pregnant hamsters. Similar observations were earlier made by Greenwald[13]. However, in the present study, a significant drop in circulating progesterone levels in day 8 and 12 pregnant animals was observed, suggesting that estradiol affected luteal functionality, although this did not apparently affect the course of pregnancy. It is conceivable that the amount of progesterone still available might be enough to prevent the termination of pregnancy. Administration of estradiol resulted in lowered serum progesterone levels, without at the same time interfering with serum LH levels or the ability of the luteal tissue to bind labelled hCG. These observations suggest that estradiol might be directly acting on the corpus luteum to block progesterone output.

4.3. *Effect of estradiol* in vitro *on the luteal functionality*

The results of the present study indicate that in the hamster, estradiol can inhibit progesterone secretion by corpus luteum *in vitro*. Corpora lutea of day 8 pregnant animals appeared to be more sensitive than corpora lutea of either day 12 or 15, to exogenous estradiol. Whereas a dose of 0·5 μg/ml of estradiol could cause significant inhibition in progesterone secretion by day 8 corpora lutea, the same dose was found to be not effective in the case of day 12 or 15 corpora lutea. Thus, it is apparent that the ability of estradiol to inhibit progesterone secretion is a function of luteal age. One possible explanation for the refractoriness of day 12 and 15 corpora lutea might be the high endogenous levels of estrogen present during late pregnancy. It appears possible that the level of estrogen present in the ovarian mileu is already high in 12 and 15 day pregnant animals and the luteal functionality is already affected as evidenced by the decreased secretion of progesterone by 12 and 15 day pregnant corpora lutea compared to day 8 corpora lutea. Therefore, exogenous estradiol will have maximum effect on day 8 corpora lutea and will not manifest additional effects with day 12 and 15 corpora lutea.

The minimum concentration (0·5 μg/ml) of estradiol which blocked progesterone secretion in this *in vitro* model far exceeded the concentration of the steroid *in vivo*. However, it is difficult to assess the estrogen concentration in the local ovarian circulation *in vivo*, which can be much higher than that detected at a gross level. Secondly, a gradual increase in concentrations *in vivo* over periods of time may bring about this effect, which perhaps can be brought about *in vitro* in short term experiments only by a much higher concentration of estradiol.

Though it was found that repeated washing with fresh medium of the corpora lutea pretreated with estradiol did not reverse its ability to reduce progesterone secretion, (data not shown) the fact that LH could stimulate progesterone secretion in presence of estradiol to some extent suggested that estradiol did not cause irreversible derangement in

the steroidogenic machinery of the cell. However, the responsiveness of corpora lutea to LH in presence of estradiol diminished considerably.

The suggestion that estradiol, in addition to affecting the secretion, might also inhibit synthesis was examined by incubating corpora lutea with or without estradiol for different time intervals and estimating progesterone in the tissue. A difference in progesterone levels between the control and treated tissue was not observed at any time point, leading to the suggestion that estradiol may not be blocking progesterone synthesis (data not shown). On the other hand, if estradiol blocks secretion alone, an accumulation of progesterone in the corpora lutea treated with estradiol should normally occur. However, the results obtained appear to be contrary to this assumption. In view of the observation that the final conversion of pregnenolone to progesterone by 3–β–OH steroid dehydrogenase-isomerase complex is inhibited by progesterone[13], the effect of estradiol may therefore be related to end–product inhibition. If a block in secretion occurs, intracellular progesterone levels may increase to a level where progesterone synthesis is inhibited by the rapidly increasing intracellular progesterone. That this process might be quite rapid and the increase in intracellular progesterone levels might be transient is suggested by experiments wherein even at a time point of 15′, accumulation of progesterone in the luteal tissue treated with estradiol could not be demonstrated (data not presented).

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