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In vivo and in vitro Ovarian Steroidogenesis in the Pregnant Rat

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ABSTRACT

The interrelationship between follicular and luteal function during pregnancy in the rat was examined by in vivo and in vitro methods. Corpora lutea (CL) and nonluteal ovarian tissues (NLO) were removed on Days 2 to 22 (at 2 day intervals) (day sperm-positive = Day 1 of pregnancy), and incubated separately for 2 h to determine the production rate of steroids. Changes in progesterone (P), 20α-dihydroprogesterone (20α-OHP), testosterone (T), and estradiol-17β (E_2) in peripheral blood, CL, and NLO, and levels of LH and FSH in serum and pituitary were measured by radioimmunoassay.

A marked decline in the in vitro production rate of E_2 and T in NLO occurred between Days 14 and 18 followed by an abrupt increase on Days 20 and 22. These changes correlated with the levels of serum LH between Days 14 and 22. Serum FSH also declined on Day 16 but returned to basal levels by Day 18. Despite the fall in NLO production of E_2, serum E_2 started to increase on Day 14 with a progressive rise continuing until term. A marked increase in luteal content of E_2 occurred on Day 16 and continued until Day 22, whereas in vitro production of E_2 by CL declined from Day 14 onward.

Serum T also began to increase on Day 14 with the peak attained between Days 18 and 20, paralleling the rising E_2 levels. In vitro production of luteal T increased on Day 12 with peak values present between Days 16 and 20, paralleling the pattern of serum T.

Maximal values of serum P occurred between Days 14 and 16 and decreased after Day 18. In vitro production of luteal P increased abruptly on Day 4, with peak values on Day 10 and a decline by Day 16. There was a second peak in in vitro production of P on Day 22, although serum and luteal levels of P were already low. Changes in serum levels of 20α-OHP and its in vitro production rate were inversely related to changes in P throughout pregnancy.

These findings indicate that at the end of pregnancy the CL produce large amounts of P in vitro after functional luteolysis has occurred in vivo. The high levels of P during midpregnancy may be involved in the suppression of follicular maturation, probably by lowering basal levels of serum LH. Furthermore, between Days 14 and 18, secretion of T and E_2 represents proportionally more luteal than follicular sources of the hormones in the pregnant rat.

INTRODUCTION

During midpregnancy in the rat, the marked increase in ovarian progesterone (P) secretion coincides with increasing size of the corpora lutea (CL) (Eto et al., 1962; Fajer and Barraclough, 1967; Uchida et al., 1970; Ichikawa et al., 1974). In contrast, all large antral follicles degenerate during midpregnancy and do not reappear until the end of pregnancy (Greenwald, 1966; Taya and Sasamoto, 1977). The exact mechanism for this suppression of follicular development remains to be determined. Despite the great deal of work which has been done on ovarian function during pregnancy in the rat, relatively little attention has been directed towards correlating serum steroid levels either with the steroidogenic capacity of the ovary in toto or with the capacity of the CL and the nonluteal ovarian tissue (NLO) to secrete steroids. The present paper was therefore undertaken to provide both a comprehensive account of changes in ovarian steroidogenesis throughout pregnancy in the rat by determining estradiol-17β (E_2), testosterone (T), P, and 20α-dihydroprogesterone (20α-OHP) in

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serum and in isolated CL and NLO, and a concurrent assessment of the in vitro steroidogenic capacity of these ovarian compartments.

MATERIALS AND METHODS

Adult female rats of the Holtzman strain, 200–270 g, were maintained on a 14L:10D schedule (lights on at 0500 h). Vaginal smears were examined daily. At about 1700 h on the day of proestrus, each female was transferred to the cage of a single male and left overnight. Mating was verified the next morning by the presence of sperm in the vaginal smear; this was designated as Day 1 of pregnancy. Parturition consistently occurred on Day 23 of pregnancy. Groups of rats were decapitated between 0900 and 1000 h on each of Days 2–22 (at 2 day intervals). Pregnancy was confirmed in rats killed on Days 1–6 by the presence of embryos in oviductal or uterine flushings and after Day 8 by the presence of implantation sites. Blood was collected and serum saved for radioimmunoassay (RIA) of LH, FSH, P, 20α-OHP, T, and E_2. Anterior pituitary glands removed immediately after death were homogenized in 4 ml cold saline (8.5 g NaCl/liter). After centrifugation, the supernatant fraction was saved for RIAs of LH and FSH.

In vitro Incubation

Details of the incubation technique were described by Terranova et al. (1978). Immediately after blood collection, the ovaries were removed, placed in ice-cold saline, and all CL were dissected and cleaned of adhering NLO tissue. After weighing, five CL (4.5–28.7 mg) and the resultant NLO (14.8–25.2 mg) from the same animals were placed in 0.4 ml 95% ethanol for zero time steroid determinations or in an incubation vial containing 1.0 ml freshly gassed (95% O_2:5% CO_2) Krebs-Ringer bicarbonate (KRB) buffer. The tissues were then incubated for 2 h at 37°C in a shaker bath. At the end of the incubation, the medium was snap-frozen in an alcohol-solid CO_2 mixture and stored at −20°C; the tissues were washed gently with cold saline and stored in 0.4 ml 95% ethanol. All tissues were homogenized in 95% ethanol and stored at −20°C until used for estimation of steroids. Production rate was expressed as pg or ng steroid/CL incubated/h for CL and pg or ng steroid/mg NLO incubated/h for NLO and was defined as

$$\text{Production rate} = \frac{\text{Final content (concentration) (tissue + medium)} - \text{Initial content (concentration) (tissue)}}{2\text{ h}}$$

Since the growth of the CL of pregnancy in the rat represents hypertrophy rather than hyperplasia, we elected to present the production rates as steroids per CL rather than as steroids per mg/CL. However, with the exception of testosterone, the *patterns* of steroid production rate were identical regardless of which way they were expressed.

Gonadotropin RIAs

RIA kits for determination of levels of serum and pituitary LH and FSH were provided by NIAMDD. Methods of these assays were similar to those of Bast and Greenwald (1974). Antisera used were anti-rat LH serum 3 and anti-rat FSH serum 9. Reference preparations used for standards were rat LH-RP-1 (0.03 × NIH-LH-S1), FSH-RP-1 (2.1 × NIH-FSH-S1). Iodinated preparations were rat LH-I-1 and FSH-I-1. In the LH and FSH assays 200 μl were assayed, whereas in the PRL assay 25 μl and 50 μl were assayed. Duplicate serum and pituitary samples were assayed.

Steroid RIAs

The RIAs for steroids were the same as described previously (Terranova et al., 1978; Terranova and Greenwald, 1978) using P antisera (Surve et al., 1976). T antisera (Pang and Johnson, 1974), and E_2 antisera (Exley et al., 1971). Since T antiserum crossreacts with 5α-dihydrotestosterone (58%), androstenedione (2%), and progesterone (0.07%), the steroid levels are referred to as testosterone-equivalents. The antiserum to 20α-OHP was supplied by Drs. C. N. Pang and J. Hilliard (University of California at Los Angeles), and the only steroid which crossreacted (>1%) with the antiserum was 20β-dihydroprogesterone (42%). The lower limits of sensitivity in the assay for P, 20α-OHP, T, and E_2 were 5 pg, 5 pg, 2 pg, and 2 pg per tube, respectively.

Statistics

Data were analyzed using Duncan's multiple range test (Steel and Torrie, 1960). Differences were judged significant if $P<0.05$.

RESULTS

Tissue Weights

The mean weight of the CL gradually increased from Days 2 (1.9 ± 0.02 mg) to 12 (2.2 ± 0.1 mg) of pregnancy, then started to increase markedly on Day 14 (3.6 ± 0.3 mg) with the maximal value reached on Day 20 (5.7 ± 0.2 mg). The mean weight of the NLO gradually decreased from Days 2 (23.7 ± 1.3 mg) to 20 (14.8 ± 1.4 mg) of pregnancy with the lowest value observed on Day 20, followed by a significant increase on Day 22 (20.4 ± 1.0 mg).

Estradiol-17β (Fig. 1)

Serum E_2 levels were unchanged from Days 2 through 12 and then increased gradually until Day 22. Luteal content of E_2 increased from Days 2 through 14, followed by an abrupt increase by Day 16. The high content of luteal E_2 was maintained until Day 20, with a further increase on Day 22. There was a significant rise in nonluteal ovarian concentration of E_2 on Day 4; the concentration was relatively stable until Day 12, followed by a precipitous decrease between Days 14 and 16. After Day 18, concen-

tration of nonluteal E_2 increased abruptly with the highest value reached on Day 22.

A significant increase in the production rate of E_2 by CL occurred on Day 4 with a further increase on Day 6; the production rate of E_2 decreased by Day 14 and remained relatively constant until Day 22.

The production rate of E_2 by NLO significantly increased on Day 4 with the first peak reached on Day 8, with values about 10 times higher than on Day 2. The production rate of nonluteal E_2 abruptly declined by Day 16 but sharply increased again from Days 18 to 22.

Testosterone (Fig. 2)

Serum T remained unchanged from Days 2 to 12 and then increased progressively until Day 18, followed by a significant decline by Day 22. The first significant rise in luteal content of T occurred on Day 4, and the levels were maintained until Day 12, followed by a slow increase until Day 22. From Days 2 to 12 of pregnancy the concentration of T in NLO was relatively constant, but the levels decreased significantly between Days 14 and 18 with the nadir reached on Day 16. The concentration of T began to rise on Day 20, and the levels increased abruptly on Day 22.

The production rate of luteal T increased significantly on Day 4, then decreased slowly by Day 10. The values began to rise on Day 12, reaching peak values between Days 16 and 20, followed by a significant decline by Day 22. The production rate of T by NLO was fairly constant from Days 2 through 10; from then until Day 16, a marked decrease occurred. By Day 16, the production rate by NLO was at its lowest and then sharply increased from Days 18 to 22.

Progesterone (Fig. 3)

Serum levels of P reached the first peak on Day 6 and the levels further increased from Day 12 to the second peak on Day 16. An abrupt

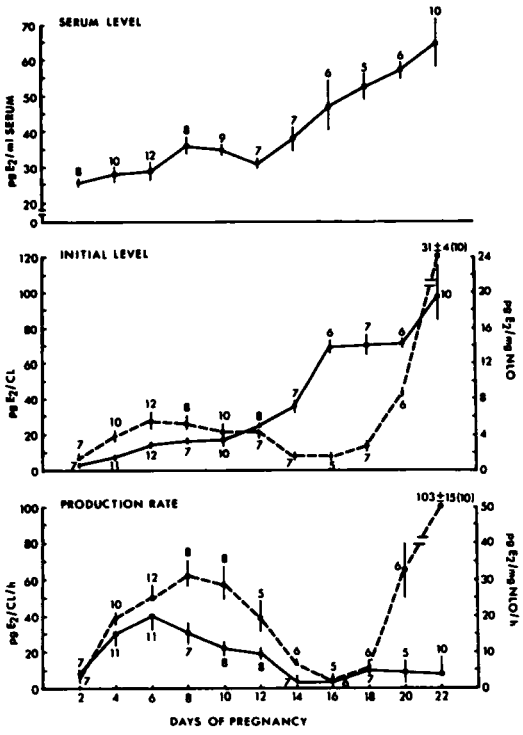

FIG. 1. Levels of E_2 in serum, CL, and NLO and in vitro production rates of E_2 by CL or NLO at specified days during pregnancy in the rat. Top panel) concentrations of serum E_2. Middle panel) luteal (—) and nonluteal (- - -) levels of E_2. Lower panel) in vitro production rates of E_2 by CL (—) or NLO (- - -). In all figures, results are expressed as mean ± SEM, with number of animals indicated for each point.

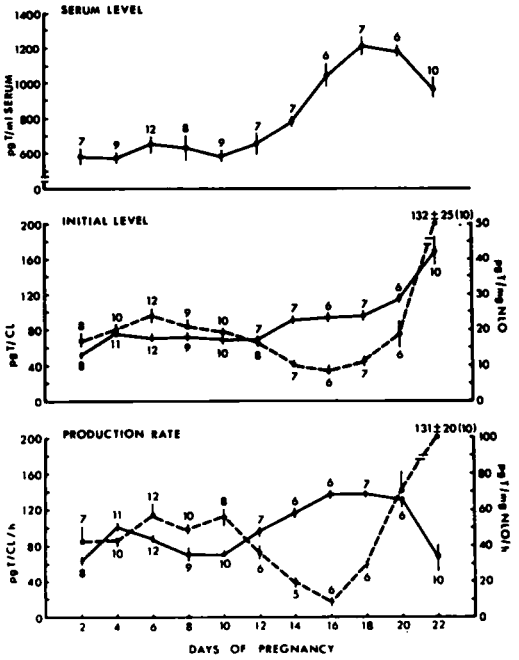

FIG. 2. Levels of T (equivalents) in serum, CL, and NLO and in vitro production rates of T by CL or NLO at specified days during pregnancy. Top panel) concentrations of serum T. Middle panel) luteal (—) and nonluteal (- - -) levels of T. Lower panel) in vitro production rates of T by CL (—) or NLO (- - -).

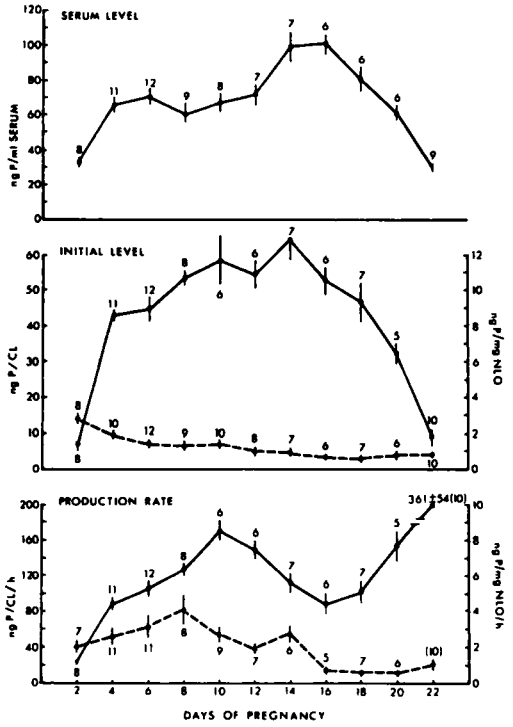

FIG. 3. Levels of P in serum, CL, and NLO and in vitro production rates of P by CL or NLO at specified days during pregnancy. Top panel) concentrations of serum P. Middle panel) luteal (—) and nonluteal (- - -) levels of P. Lower panel) in vitro production rates of P by CL (—) or NLO (- - -).

fall began after Day 18. Luteal content of P continued to increase until Day 14. Thereafter, the content of CL decreased sharply by Day 22. The concentration of nonluteal P was negligibly low compared with the CL, with the highest NLO concentration on Day 2; the lowest value was reached on Day 18.

A significant rise in the production rate of P by CL occurred on Day 4, and the values continued to increase until Day 10, followed by a decline until Day 16. The production rate of P by CL increased again with the highest value reached on Day 22. The NLO produced in vitro a negligible amount of P throughout pregnancy compared with the CL. However, when production rate of P by NLO during the first 14 days of pregnancy was compared with values on Days 16 through 22, a significant difference was evident.

20α-Dihydroprogesterone (Fig. 4)

Serum levels of 20α-OHP decreased gradually from Days 2 to 8 and remained low until Day 20. A significant increase in serum 20α-OHP was noted on Day 22. Luteal content of 20α-OHP increased significantly from Days 2 to 4. The levels were unaltered until Day 12, then began to rise on Day 14 and peaked on Day 22.

From Days 2 to 10 the in vitro production rate of 20α-OHP was relatively low, but the values began to rise on Day 12, followed by a striking increase by Day 16. A high production rate was sustained until Day 20, followed by a sharp drop by Day 22.

Changes in in vitro Luteal Steroidogenesis During Pregnancy (Table 1)

In vitro production rates of steroids were calculated as either per CL or mg/CL to determine whether the hormone profiles differed

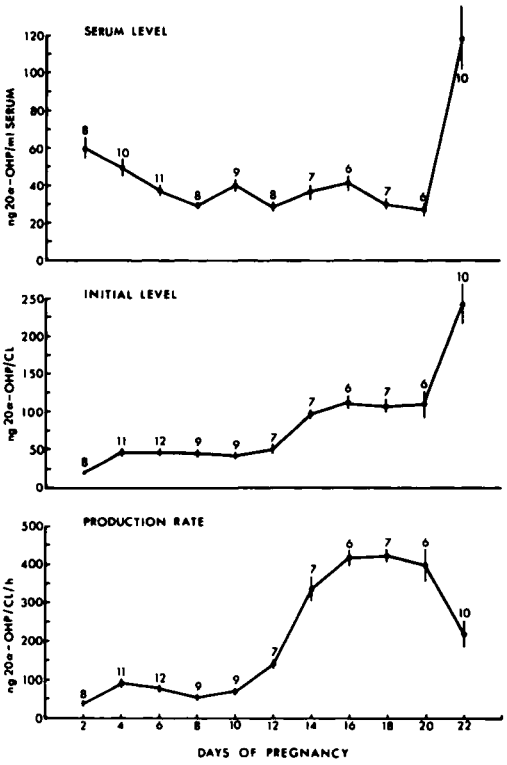

FIG. 4. Levels of 20α-OHP in serum and CL and in vitro production rates of 20α-OHP by CL at specified days during pregnancy. Top panel) concentrations of serum 20α-OHP. Middle panel) luteal levels of 20α-OHP. Lower panel) in vitro production rates of 20α-OHP by CL.

TABLE 1. In vitro production rates of P, 20α-OHP, T, and E_2 by CL at specified days during pregnancy (mean ± SEM).

Day of pregnancy	Determinations	Hormone	Production rate[a] (ng or pg/CL/h)	Production rate[a] (ng or pg/mg CL/h)
2	8	P	21.9 ± 2.4	22.2 ± 2.2
	8	20α-OHP	38.0 ± 5.6	42.2 ± 4.8
	8	T	62.9 ± 4.1	72.7 ± 4.7
	7	E_2	8.2 ± 1.5	9.2 ± 1.7
4	11	P	88.2 ± 7.2	61.9 ± 4.8
	11	20α-OHP	90.8 ± 9.7	57.3 ± 5.7
	11	T	89.9 ± 4.2	57.6 ± 2.6
	11	E_2	29.5 ± 6.0	16.1 ± 2.2
6	12	P	104.3 ± 8.8	65.8 ± 5.1
	12	20α-OHP	76.8 ± 5.7	46.7 ± 3.6
	12	T	88.0 ± 2.9	53.8 ± 1.7
	11	E_2	39.6 ± 7.5	24.6 ± 4.0
8	8	P	125.0 ± 8.2	73.8 ± 5.4
	9	20α-OHP	52.3 ± 6.5	31.9 ± 4.7
	9	T	69.9 ± 5.7	42.0 ± 3.3
	7	E_2	30.2 ± 7.0	14.5 ± 3.0
10	6	P	169.6 ± 12.7	92.2 ± 8.5
	9	20α-OHP	69.4 ± 8.2	34.8 ± 3.4
	10	T	69.5 ± 2.7	38.1 ± 2.1
	8	E_2	24.4 ± 4.9	12.0 ± 2.9
12	6	P	147.3 ± 9.5	59.2 ± 4.7
	7	20α-OHP	138.9 ± 10.8	59.4 ± 4.7
	7	T	94.5 ± 4.4	40.5 ± 3.4
	8	E_2	19.0 ± 4.0	6.6 ± 1.7
14	7	P	111.7 ± 11.6	30.4 ± 4.3
	7	20α-OHP	334.4 ± 35.3	95.8 ± 8.4
	6	T	116.0 ± 4.5	32.0 ± 2.2
	7	E_2	2.9 ± 0.2	0.8 ± 0.1
16	6	P	88.8 ± 11.6	18.6 ± 2.5
	6	20α-OHP	412.0 ± 20.7	86.4 ± 5.1
	6	T	135.8 ± 4.0	28.5 ± 0.9
	6	E_2	2.5 ± 1.3	0.5 ± 0.3
18	7	P	104.5 ± 13.2	19.4 ± 2.2
	7	20α-OHP	417.3 ± 26.4	78.0 ± 4.2
	7	T	134.6 ± 1.4	24.8 ± 0.8
	7	E_2	10.5 ± 4.0	1.9 ± 0.7
20	5	P	154.9 ± 17.1	27.4 ± 3.9
	6	20α-OHP	398.2 ± 41.3	70.5 ± 6.7
	6	T	130.7 ± 8.2	23.6 ± 1.4
	5	E_2	9.0 ± 2.5	1.6 ± 0.4
22	10	P	360.5 ± 54.2	81.4 ± 9.9
	10	20α-OHP	215.2 ± 56.8	49.1 ± 9.0
	10	T	65.6 ± 10.6	15.9 ± 2.2
	10	E_2	8.2 ± 3.8	1.9 ± 0.9

[a] P and 20α-OHP are expressed as ng whereas T and E_2 are expressed as pg.

when expressed in different ways (Table 1). The patterns of production rate for P, 20α-OHP, and E_2 were the same when the results were expressed either way. However, the increase in the production rate of T between Days 12 and 20, when the results were expressed per CL, was not observed when the results were expressed on the basis of CL weight.

Serum and Pituitary Levels of LH and FSH (Fig. 5)

Serum concentrations of LH significantly increased from Day 2 to Day 4, and remained relatively constant until Day 10; from then until Day 18, a gradual decrease occurred. On Day 18, serum LH was at its lowest value and then abruptly increased on Days 20 and 22. Serum FSH concentrations remained relatively constant throughout pregnancy except on Day 16. Serum FSH fell from Day 14 to 16, but increased again on Day 18. Pituitary content of LH and FSH increased with time, and reached maximal levels between Days 16 and 18, followed by a slight decline thereafter. The maximal pituitary content of LH and FSH during pregnancy was higher than at any stage of the estrous cycle.

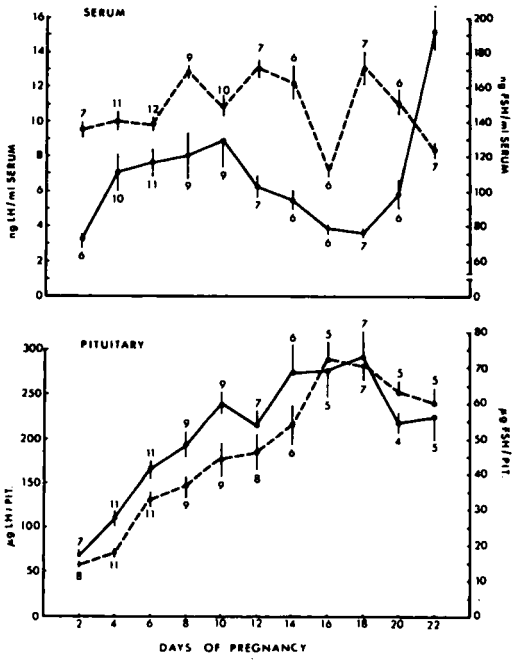

FIG. 5. Serum and pituitary levels of LH (—) and FSH (- - -) during pregnancy in the rat.

DISCUSSION

A significant increase in peripheral E_2 began on Day 14 and continued to term whereas a significant increase in atresia of large antral follicles occurred between Days 14 and 18 (Greenwald, 1966; Taya and Sasamoto, 1977). The present results on peripheral E_2 levels agree with previous findings that a reduced secretion of E_2 during midpregnancy was not observed in rat ovarian venous plasma, using bioassay (Yoshinaga et al., 1969) or RIA (Shaikh, 1971). In the present study, the limited ability of NLO to secrete E_2 and T during midpregnancy correlates with the absence of large antral follicles. The marked decrease in E_2 and T and in vitro production of these hormones in NLO between Days 14 and 18 coincided with the decreasing secretion of LH (Fig. 5). During Days 14–18 of pregnancy, maximal values of serum P correlated with minimal values for serum LH. This suggests that the basal serum levels of LH between Days 14 and 18 are insufficient to support optimally thecal cell synthesis of androgen and thus follicular E_2 secretion; however, a direct inhibitory action of P on follicular cell function cannot be excluded. Taya and Greenwald (1981a) showed that exogenous P injected into the diestrous rat inhibited follicular E_2 and T secretion within 1 h by lowering serum LH. The decrease in serum FSH on Day 16 (Fig. 5) may also affect follicular maturation and maintenance of follicular activity, probably via inhibition of the activity of aromatizing enzymes in the granulosa cells.

Beginning on Day 20, the increase in the concentration of E_2 and T and in vitro production of these hormones by NLO paralleled increasing secretion of LH (Fig. 5). Richards and Kersey (1979) clearly demonstrated that injection of LH antiserum between Days 20–22 of pregnancy abolished estradiol accumulation by follicles incubated in the absence but not in the presence of T. The serum levels of LH (Fig. 5) agree in general with those of previous papers (Brown-Grant et al., 1972; Morishige et al., 1973; Cheng, 1976) but contrast with those reported by Linkie and Niswender (1972). The changes in serum FSH during pregnancy in rats in our study differ from previous findings (Linkie and Niswender, 1972; Cheng, 1976).

The increase in luteal contents of E_2 on Day 16 correlated with changes in peripheral E_2

levels, although in vitro production of the hormone by CL declined on Day 14. Previous papers have established that in the second half of pregnancy in the rat the CL actively aromatize androgens to estrogens both in vivo (Gibori and Kraicer, 1973) and in vitro (Elbaum and Keyes, 1976), and may play an important role in the regulation of luteal function in the rat (Gibori and Keyes, 1978).

Whether there is a surge in serum T during pregnancy is still controversial because of contradictory results. Weizenbaum et al. (1978) reported that serum T concentrations rose significantly above cyclic levels on Days 1–2 and on Days 10–14. On the other hand, Gibori et al. (1979) reported that the lowest levels of peripheral androgen were reached on Days 8–9 of pregnancy and started to rise at midpregnancy, with peak levels on Days 17 and 18 and a decline thereafter. In our studies, high concentrations of serum T were observed between Days 16 and 20 of pregnancy. This elevation of serum T agrees, in part, with the finding of Gibori et al. (1979) whereas the low levels of serum T in the first half of pregnancy were not observed. However, the source of androgen during pregnancy remains unclear. Gibori et al. (1979) reported that there are no significant differences in the androgen concentration of CL throughout pregnancy, and a highly significant increase in androgen was found in extraluteal tissue of the ovary on Day 22 of pregnancy only. Therefore, they suggested that serum androgen is from an extraovarian source, probably the feto-placental unit. The rat placenta has a high activity of 3β-hydroxysteroid dehydrogenase (Wiener, 1974), but lacks aromatizing enzymes (Sybulski, 1970). Sanyl and Villee (1973) have demonstrated the predominance of 5α-reduced metabolites by the rat placenta when incubated in vitro. Chan and Leathem (1975) have further shown that androstendione, as well as testosterone, was detected in rat placenta homogenate and its minced preparations. Therefore, it is possible that the rat placenta can convert C_{19} steroids from C_{21} precursors and may have an important physiological significance, serving as a source of substrates for estrogen formation by the ovary. In the present study, however, luteal T content started increasing at Day 14 and reached peak levels on Day 22. This increase in luteal T levels corresponded to changes in luteal E_2 content. In addition, changes in luteal T production in vitro correlated with changes in serum T. These results indicate that CL of pregnancy can secrete considerable amounts of T in the second half of pregnancy both in vivo and in vitro.

After Day 18, the CL undergo functional luteolysis, and serum and luteal levels of P decline precipitously (Fig. 2) coincident with increases in serum LH (Fig. 5). In the present study, serum concentrations of P and 20α-OHP during the first half of pregnancy are well correlated with the contents and in vitro production of these hormones in the CL. However, peripheral levels of 20α-OHP on Days 20–22 show an inverse relationship to in vitro production rates of the hormones by the CL. Our data also indicate that in vitro luteal P production on Days 20 and 22 consists of a marked increase in the synthesis of P rather than the release of P into the incubation media. The reason for the inconsistency between in vitro and in vivo luteal P and 20α-OHP secretion during the second half of pregnancy is not clear. However, pituitary gonadotropin, probably LH, may be involved in this phenomenon, since CL obtained on Day 22 from pregnant rats hypophysectomized on Day 12 produced less P in vitro than did CL from sham-hypophysectomized animals (Taya and Greenwald, 1981b).

There is a striking increase in concentration of cholesterol in the CL on Day 18 of pregnancy with the elevated level maintained until the end of pregnancy (Uchida et al., 1970), indicating that the CL at term contain an adequate amount of precursors for steroid biosynthesis. Similarly, following the in vivo administration of antiluteinizing hormone to pregnant rats, there is a drastic fall in serum P, and yet the CL in vitro synthesize greater amounts of P than controls (Behrman et al., 1972; Terranova and Greenwald, 1981). The most likely explanation for the paradoxical results is that LH deprivation increases free and esterified cholesterol in the CL, and the mass action effect of the sterol precursor available for conversion to progesterone is manifested in vitro (Behrman et al., 1972). Furthermore, in the CL of pregnancy, there is an increase in acid phosphatase (indicative of degeneration) only *after* parturition (Lobel et al., 1961).

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