Initiation of follicular maturation during mid-pregnancy by the administration of LH in the rat

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Summary. Pregnant rats were injected twice daily for 1–3 days (Days 13–16 of pregnancy) with various doses of ovine LH. Follicular maturation was determined by the ability of the follicles to ovulate in response to 10 i.u. hCG as well as by endogenous production of oestradiol-17 β and inhibin. In control animals, no ovulation was induced by hCG given on Day 16 of pregnancy. An injection of hCG on Day 16 of pregnancy, however, induced ovulation in LH-treated animals (6.25–50.0 μ g LH per injection, s.c. at 12-h intervals from Days 13 to 16). Concentrations of oestradiol-17 β and inhibin activity in ovarian venous plasma increased after the administration of LH, indicating that development of ovulatory follicles had been induced. Abolishing the decline in plasma LH values therefore induced maturation of a new set of follicles or prevented the atresia of large antral follicles usually seen at this time of pregnancy. Plasma and pituitary concentrations of FSH decreased in LH-treated animals compared with those in control animals. Concentrations of progesterone, testosterone and oestradiol-17 β in the peripheral plasma were not significantly different between the two groups. These results suggest that the increase in inhibin secretion from the ovary containing maturing follicles after LH treatment may suppress the secretion of FSH from the pituitary gland. These findings indicate that (1) the development of ovulatory follicles can be induced by the administration of exogenous LH during mid-pregnancy in the rat and (2) basal concentrations of FSH are enough to initiate follicular maturation even in the presence of active corpora lutea of pregnancy, when appropriate amounts of plasma LH are present.

Keywords: pregnancy; inhibin; follicle; FSH; LH; rat

Introduction

The anovulatory state during pregnancy in the rat is characterized by the persistence of functional corpora lutea and interruption of cyclic ovulation. During pregnancy in the rat, striking changes in the ovary occur, i.e. the corpora lutea of pregnancy become larger and the secretion of progesterone and oestradiol-17 β is altered. Maximal values of serum progesterone are noted during mid-pregnancy, followed by a decrease at the end of pregnancy, while serum oestradiol-17 β concentrations increase during mid-pregnancy and rise progressively until term (Taya & Greenwald, 1981). All large antral follicles degenerate during mid-pregnancy in the rat and reappear at the end of pregnancy, corresponding to the changes in serum concentration of LH (Greenwald, 1966; Taya & Sasamoto, 1977; Bogovich *et al.*, 1981; Taya & Greenwald, 1981; Osman, 1986; Miyamoto *et al.*, 1987). If the fall in plasma LH concentrations during mid-pregnancy underlies the degeneration of large antral follicles, then abolition of this decline by treatment with LH, which maintains plasma LH concentrations at values similar to or higher than those pertaining before the decline, should induce maturation of follicles by recruiting new follicles and/or preventing atresia of large antral follicles.

The present study was undertaken to examine whether the development of ovulatory follicles can be induced by the administration of exogenous LH during mid-pregnancy in the rat.

Materials and Methods

Animals. Adult female rats of the Wistar strain were maintained under controlled conditions of temperature (23–27°C) and lighting (lights on 05:00–19:00 h). Vaginal smears were examined daily and on the day of pro-oestrus each female was transferred at about 17:00 h to the cage of a single male rat and left overnight. Mating was verified the next morning by the presence of spermatozoa in the vaginal smear; this was designated as Day 1 of pregnancy. Usually parturition consistently occurred on Day 22 of pregnancy in our colony of rats.

LH treatment. Various doses of ovine LH (NIADDK o-LH-24) dissolved in 50% (w/v) polyvinylpyrrolidone (PVP) were injected s.c. twice daily at 12-h intervals from 08:00 h on Day 13 to 08:00 h on Day 16 of pregnancy. The ability of any antral follicles to ovulate after LH treatment was determined by injecting 10 i.u. human chorionic gonadotrophin (hCG, 2200 i.u./mg; Sankyo Zoki Co., Tokyo, Japan) dissolved in 0.2 ml 0.85% (w/v) NaCl into the tail vein under ether anaesthesia. All injections of hCG were given 9 h after the last LH injection. Animals were killed 18–20 h after the injection of hCG and the oviducts were examined for oocytes.

Plasma concentrations of immunoreactive LH after injection of sheep LH in hypophysectomized and ovariectomized rats. Three doses of LH (NIADDK o-LH-24; 25, 50 and 100 µg/injection) dissolved in 0.2 ml 50% PVP were injected s.c. in hypophysectomized and ovariectomized rats 5 days after the surgery. At various times after the treatment, individual blood samples were collected from the jugular vein (0.5 ml) of 5 rats per group under ether anaesthesia. Individual plasma samples were obtained after centrifugation, and stored at –20°C until assayed for LH.

Plasma concentrations of FSH, progesterone, testosterone and oestradiol-17β after LH treatment. LH (50.0 µg/injection) dissolved in 0.2 ml 50% PVP was injected s.c. at 12-h intervals from 08:00 h on Day 13 to 08:00 h on Day 16 for a total of 7 injections. At various times after the start of treatment, individual blood samples were collected from the jugular vein (1.0 ml) of 5 rats per group under ether anaesthesia. Blood samples of each rat were taken on 2 occasions with an interval of 42 h. Individual plasma samples were obtained after centrifugation, and stored at –20°C until assayed for FSH. Individual blood samples (2 ml) were collected in the same manner at 08:00 h on Days 13–16 of pregnancy from another group of rats for measurement of progesterone, testosterone and oestradiol-17β. Concentrations of endogenous LH could not be detected, since the sheep LH cross-reacted strongly with rat LH in the rat LH radioimmunoassay system of NIADDK.

Concentrations of inhibin activity in ovarian venous plasma after LH treatment. At 17:00 h from Days 13 to 16 of pregnancy, rats, which had received vehicle or LH (50.0 µg/injection in 0.2 ml PVP) at 12-h intervals from 08:00 h on Day 13, were anaesthetized with pentobarbitone sodium (30 mg/kg body weight, i.p.) and ovarian venous blood was collected for 10 min by the method of Eto *et al.* (1962). Plasma was separated by centrifugation and stored at –50°C until assayed for inhibin.

Pituitary contents of FSH and LH after LH treatment. Immediately after collection of ovarian venous blood on Day 16 of pregnancy, anterior pituitary glands were removed and homogenized in 4 ml cold (4°C) 0.85% (w/v) NaCl solution. After centrifugation at 36 000 g for 30 min at 4°C, the supernatant fraction was obtained and stored at –20°C until assayed for FSH and LH.

Radioimmunoassay of FSH, LH, progesterone, testosterone and oestradiol-17β. Concentrations of FSH in peripheral plasma and contents of FSH and LH in pituitary glands were measured using the NIADDK radioimmunoassay kits for rat FSH and LH. Iodinated preparations were rat FSH-I-5 and LH-I-5. The antiserum used was anti-rat FSH-S-11 for FSH radioimmunoassay. In the LH radioimmunoassay, anti-rat LHS-8 was used for measurement of pituitary contents, whereas anti-ovine LH (GDN 15) was used for measurement of plasma concentrations of LH of hypophysectomized and ovariectomized rats after the injection of ovine LH. Antiserum against ovine LH (GDN 15) was provided by Dr G. D. Niswender (Colorado State University, Fort Collins, CO, U.S.A.). Results were expressed in terms of NIADDK rat FSH-RP-1 and LH-RP-1. The sensitivity of the assay for LH and FSH was 0.25 and 1.2 ng/tube and the intra- and interassay coefficients of variation were 3.7 and 10.9% for FSH, 4.2 and 10.5% for LH, respectively.

Concentrations of progesterone, testosterone and oestradiol-17β in peripheral and ovarian venous plasma were determined by double-antibody radioimmunoassay systems using ¹²⁵I-labelled radioligands as described previously (Taya *et al.*, 1985). Antisera against progesterone (GDN 337; Gibori *et al.*, 1977), testosterone (GDN 250; Gay & Kerlen, 1978) and oestradiol-17β (GDN 244; Korenman *et al.*, 1974) were provided by Dr G. D. Niswender. The sensitivities of the assays for progesterone, testosterone and oestradiol-17β were 0.625, 0.625 and 0.3125 pg/tube, respectively. The intra- and interassay coefficients of variation were 2.6 and 12.0% for progesterone, 4.8 and 9.6% for testosterone and 5.8 and 11.4% for oestradiol-17β, respectively.

Bioassay of inhibin activity. After removal of steroids by charcoal treatment, pooled samples of ovarian venous plasma (5 animals/group) were assessed for FSH-inhibiting activity (inhibin) using the dispersed anterior pituitary cell

bioassay system (Kimura *et al.*, 1983), partly modified as described previously (Sasamoto *et al.*, 1987). Measurement of inhibin activity was undertaken as a parallel line assay of the 2×2 point system using the method of Finney (1964) and the potency of inhibin activity was expressed as relative units compared with the pooled ovarian venous plasma at 08:00 h on Day 13 of pregnancy (reference standard). One unit was defined as the activity of 1.0 ml pooled ovarian venous plasma at 08:00 h on Day 13 of pregnancy. The index of precision was $<21\%$ for the assay and 12.5 μ l ovarian venous plasma at 17:00 h of Day 13 showed a significant decrease in FSH amounts in the medium of the pituitary cell culture system as compared with controls.

Statistics. Significance of difference between two means was tested by Student's *t* test but when more than two means were compared, an analysis of variance was carried out and the significance of the difference between means was determined by Duncan's multiple range test (Steel & Torrie, 1960); a value of $P < 0.05$ was considered to be significant.

Results

Induced development of ovulatory follicles after administration of LH

When hCG was injected i.v. at 17:00 h on Day 16 of pregnancy, no ovulation occurred in control animals pretreated with 0.2 ml PVP. Out of 5 LH-treated animals (3.125 μ g/injection), 1 ovulated 1 oocyte. Increasing the amount of LH led to all animals ovulating with an increasing number of oocytes in response to hCG (Table 1).

Table 1. Induction of ovulation by 10 i.u. human chorionic gonadotrophin (hCG) given i.v. at 17:00 h on Day 16 of pregnancy after administration of various doses of sheep LH from Days 13 to 16 of pregnancy

Treatment*	Rats ovulating/ rats examined	No. of oocytes/ ovulating rat†
Control	0/5	—
LH		
3.125 μ g $\times$ 7	1/5	1
6.25 μ g $\times$ 7	5/5	1.6 $\pm$ 0.2 ^a
12.5 μ g $\times$ 7	5/5	4.8 $\pm$ 1.2 ^b
25.0 μ g $\times$ 7	5/5	3.8 $\pm$ 0.2 ^a
50.0 μ g $\times$ 7	5/5	7.2 $\pm$ 1.6 ^b

Values with different superscript letters are significantly ($P < 0.05$) different (Duncan's multiple range test).

*Ovine LH (3.125–50.0 μ g/injection in 0.2 ml 50% polyvinylpyrrolidone) and 0.2 ml vehicle were injected s.c. from the morning of Day 13 to the morning of Day 16 twice daily (7 injections).

†Animals were killed 18–20 h after injection of hCG to examine the oviducts for oocytes.

‡Values are mean $\pm$ s.e.m.

To determine the time-course of development of ovulatory follicles after repeated injections of LH (50.0 μ g/injection), 10 i.u. hCG were given i.v. at various times after LH administration (Table 2). In control animals pretreated with 0.2 ml PVP, 3 out of 5 animals ovulated 2.3 ± 0.3 (s.e.m.) oocytes when hCG was injected at 17:00 h on Day 14 of pregnancy, although no ovulation occurred in response to hCG injection on Day 15 or 16 of pregnancy. However, hCG given at 17:00 h on any day from Days 14 to 16 of pregnancy induced ovulation in all animals pretreated with LH, regardless of the day of initiation of the LH injection between Days 13 and 15 of pregnancy.

Table 2. Induction of ovulation in pregnant rats by 10 i.u. human chorionic gonadotrophin (hCG) given i.v. 9 h after the last injection of sheep LH

Treatment†		Day of pregnancy of LH treatment	Day of hCG injection	Rats ovulating/rats examined‡	No. of oocytes/ovulating rats§
Vehicle	0.2 ml × 3	13–14	13	3/5	2.3 ± 0.3
	0.2 ml × 5	13–15	14	0/5	—
	0.2 ml × 7	13–16	15	0/5	—
LH	50.0 µg × 3	13–14	13	5/5	9.0 ± 1.5*
	50.0 µg × 5	13–15	14	5/5	8.0 ± 0.4
	50.0 µg × 7	13–16	15	5/5	7.2 ± 1.6
	50.0 µg × 3	15–16	15	5/5	8.5 ± 0.6
	50.0 µg × 5	14–16	15	5/5	7.7 ± 0.9

* $P < 0.05$ compared with the respective control (Student's t test).

†Ovine LH (50.0 µg/injection in 0.2 ml 50% polyvinylpyrrolidone) and 0.2 ml vehicle were injected s.c. at 08:00 and 20:00 h on each day of pregnancy.

‡Animals were killed 18–20 h after injection of hCG to examine the oviducts.

§Values are mean ± s.e.m.

Fig. 1. Plasma concentrations of immunoreactive LH in hypophysectomized and ovariectomized rats receiving a single s.c. injection of 25 (●), 50 (▼) or 100 (■) µg ovine LH or 50% polyvinylpyrrolidone (PVP; □). Ovine LH (25, 50 or 100 µg/injection in 0.2 ml 50% PVP) and PVP (0.2 ml) were injected s.c. at 11:00 h on 5 days after the surgery. Results are expressed as means ± s.e.m. for 5 animals. The dotted line of the graph represents the lower limit of the assay. Different superscripts within the same sampling time differ ($P < 0.05$ by Duncan's multiple range test).

Fig. 2. Plasma concentrations of FSH in pregnant rats receiving repeated injections of ovine LH (▼) or vehicle (▽) from Days 13 to 16 of pregnancy. Ovine LH (50.0 µg/injection in 0.2 ml 50% polyvinylpyrrolidone (PVP)) or PVP (0.2 ml) were injected s.c. twice daily from the morning of Day 13 to the morning of Day 16 of pregnancy (7 injections). Results are expressed as means ± s.e.m. for 5 animals. * $P < 0.05$ compared with the value for the respective control (Student's t test).

Concentrations of immunoreactive LH in the peripheral plasma after administration of sheep LH in hypophysectomized and ovariectomized rats (Fig. 1)

Within 1 h after the injection of sheep LH into the hypophysectomized and ovariectomized rats, immunoreactive LH was detected in peripheral circulation. When animals were treated with 25 µg LH, plasma concentrations of LH increased until 3 h and the peak value was maintained until 6 h, then decreased to basal levels by 12 h after the injection. In the LH-treated animals, the peak values and the plateau times after the treatment were increased in a dose-dependent manner.

Concentrations of FSH, progesterone, testosterone and oestradiol-17β in the peripheral plasma after administration of LH

FSH (Fig. 2). In the controls, plasma FSH did not change during the period between Days 13 and 16 of pregnancy. However, plasma concentrations of FSH in the LH-treated groups significantly decreased within 15 h after the first injection of LH and the levels remained low compared with those in the controls during the period of this study. In the LH-treated animals, significantly low concentrations of FSH in the plasma were noted from 23:00 h on Day 13 to 23:00 h on Day 14 and 11:00 h on Day 16 of pregnancy.

Fig. 3. Plasma concentrations of (a) progesterone, (b) testosterone and (c) oestradiol-17 β in pregnant rats receiving repeated injections of ovine LH (■) or vehicle (□) from Days 13 to 16 of pregnancy. Ovine LH (50.0 μ g/injection in 0.2 ml 50% polyvinylpyrrolidone (PVP)) or PVP (0.2 ml) were injected s.c. twice daily from the morning of Day 13 to the morning of Day 16 of pregnancy (7 injections). Results are expressed as means $\pm$ s.e.m. for 5 animals.

Progesterone (Fig. 3). In the control and LH-treated animals, plasma concentrations of progesterone gradually increased from Days 13 to 15 of pregnancy and decreased slightly on Day 16. Concentrations in the LH-treated animals were lower than those in the corresponding controls, but the difference was not significant.

Testosterone (Fig. 3). Plasma concentrations of testosterone in the controls and LH-treated animals did not change throughout the period of observation and no significant difference was noted between the two groups.

Oestradiol-17 β (Fig. 3). Plasma concentrations of oestradiol-17 β in the controls and LH-treated animals gradually increased from Days 13 to 16 of pregnancy. Values in the LH-treated animals

showed a tendency to increase compared with the level in the controls on Days 14 and 15 of pregnancy, but the difference was not significant.

Concentrations of progesterone, testosterone and oestradiol-17 β in ovarian venous plasma (Table 3)

On Day 16 of pregnancy, in the LH-treated group the concentration of progesterone was significantly lower and that of oestradiol-17 β was significantly higher than in the controls. There was no significant difference in the concentrations of testosterone between the two groups.

Table 3. Concentrations of progesterone, testosterone and oestradiol-17 β in ovarian venous plasma of rats at 17:00 h on Day 16 of pregnancy after the administration of LH

Treatment†	Progesterone (ng/ml)	Testosterone (pg/ml)	Oestradiol-17 β (pg/ml)
Control	4442.0 $\pm$ 715.9	4870.4 $\pm$ 557.2	377.0 $\pm$ 86.0
LH	2178.0 $\pm$ 380.8*	5449.5 $\pm$ 607.2	1242.6 $\pm$ 226.2*

Values are mean $\pm$ s.e.m. for 5 animals.

* $P < 0.05$ compared with the respective control (Student's t test).

†Ovine LH (50.0 μ g/injection in 0.2 ml 50% polyvinylpyrrolidone) and vehicle were injected s.c. from the morning of Day 13 to the morning of Day 16 of pregnancy (7 injections).

Inhibin activity in ovarian venous plasma (Table 4)

Inhibin activity in control animals decreased gradually from Days 13 to 16 of pregnancy. In LH-treated animals, on the other hand, inhibin activity in ovarian venous plasma was increased within 24 h after the first injection of LH, and there was a tendency of activity higher than that of the corresponding controls during the period of observation.

Table 4. Inhibin activity in 1.0 ml ovarian venous plasma at various times after repeated injection of LH* during midpregnancy in rats

Day of pregnancy	Control rats	LH-treated rats
13	1.0	—
14	0.527 (0.291–0.825)	1.025 (0.736–1.431)
15	0.516 (0.354–0.704)	0.751 (0.364–1.369)
16	0.501 (0.289–0.761)	0.836 (0.613–1.120)

*Animals were injected s.c. with 50.0 μ g ovine LH/injection in 0.2 ml 50% polyvinylpyrrolidone or with vehicle every 12 h from 08:00 h on Days 13–16 of pregnancy (7 injections).

Values are expressed as the potency relative to the activity in ovarian venous plasma at 17:00 h on Day 13 of pregnancy (1 unit/ml); and 95% fiducial limits are given in parentheses.

Pituitary contents of LH and FSH (Table 5)

On Day 16 of pregnancy, the pituitary content of FSH in the LH-treated group was significantly lower than in the controls, whereas there was no significant difference in the pituitary content of LH between the two groups.

Table 5. Pituitary contents of FSH and LH in rats at 17:00 h on Day 16 of pregnancy after the administration of LH

Treatment†	FSH (µg/pituitary)	LH (µg/pituitary)
Control	45.8 ± 0.8	192.7 ± 25.0
LH-treated	31.6 ± 1.4*	228.2 ± 31.2

Values are mean ± s.e.m. for 5 animals

* $P < 0.05$ compared with the respective control value (Student's *t* test).

†The animals were injected with 50.0 µg ovine LH/injection in 0.2 ml 50% polyvinylpyrrolidone or vehicle every 12 h from the morning of Day 13 to the morning of Day 16 of pregnancy (7 injections).

Discussion

The present study demonstrates that the low concentrations of plasma LH normally occurring during mid-pregnancy contribute to the suppression of follicular maturation during this time. A marked decline in plasma concentrations of LH occurs between Days 13 and 19 of pregnancy in rats (Morishige *et al.*, 1973; Taya & Greenwald, 1981). Gallo *et al.* (1985) reported that LH release is pulsatile during pregnancy in the rat and the characteristics of this release vary during different stages of gestation: 53% of the LH records in pregnant rats on Days 15–17 were non-pulsatile, showing a striking decrease as compared to those on Days 6–8, in which only 20% of LH records were non-pulsatile. There was a marked increase in mean blood LH concentrations on Day 22 of pregnancy due to an increase in LH pulse frequencies.

In the present study, increasing amounts of ovine LH injected during mid-pregnancy increased the number of follicles capable of ovulating in response to hCG. Plasma concentrations of immunoreactive LH also increased in a dose dependent manner when injected in hypophysectomized and ovariectomized rats. Although bioactivity of peripheral levels of ovine LH was not known, these results suggest that the failure of follicular maturation during mid-pregnancy in rats may be due to inadequate LH stimulation. The present results also demonstrated that LH administration stimulates ovarian secretion of inhibin and oestradiol-17β, with suppression of progesterone secretion. Ovarian secretion of testosterone did not change. The ovarian oestradiol-17β response was similar to that reported by Bogovich *et al.* (1981) and Sridaran & Gibori (1983), suggesting that follicular maturation occurred.

In the present study, administration of LH resulted in a decrease in plasma concentration and pituitary content of FSH compared with their matched controls during induced maturation of ovulatory follicles in pregnant rats. Plasma concentrations and pituitary contents of FSH also declined in LH-treated lactating rats, compared with those in control animals, during the period of follicular maturation induced by LH (Taya & Sasamoto, 1988). These results indicate that subtle increases in plasma LH activity are required for preovulatory follicular development and that plasma concentrations of FSH in pregnant rats may be sufficient to support the maturation of antral follicles. Initiation of follicular maturation without a prior increase in FSH also occurs spontaneously in pregnant rats (Richards & Kersey, 1979; Taya & Greenwald, 1981), in lactating

rats (Taya & Sasamoto, 1981) and in the cyclic rat in which ovulation is blocked by the administration of anti-LH-releasing hormone serum (Matsuzono *et al.*, 1986).

A positive relationship between the number of healthy antral follicles and inhibin activity in ovarian venous plasma has been demonstrated in rats at different reproductive stages (DePaolo *et al.*, 1979; Kimura *et al.*, 1983; Taya *et al.*, 1984; Tsukamoto *et al.*, 1986). Histological observations have shown that pregnant rats have healthy antral follicles in their ovaries during the early stages of pregnancy but all large antral follicles degenerate during mid-pregnancy and reappear at the end of pregnancy (Greenwald, 1966; Osman, 1986; Miyamoto *et al.*, 1987), corresponding to the changes in inhibin activity in ovarian venous plasma (Taya *et al.*, 1984). In the present study, increased secretion of ovarian inhibin and oestradiol-17 β after the administration of LH was evident, similar to that seen in the LH-treated lactating rat (Taya & Sasamoto, 1988). These results suggest that the decrease in concentrations of FSH in the plasma and in the pituitary gland in the LH-treated rats may be due to increased secretion of ovarian inhibin, caused by induced maturation of ovulatory follicles. Carson *et al.* (1981) have demonstrated that follicular oestradiol production is primarily dependent upon increased synthesis of aromatizable androgen by theca cells and that increased thecal androgen production requires sustained increases in serum LH activity. In addition, Carson *et al.* (1981) clearly indicated that morphological features consistent with enhanced steroidogenic activity were evident in theca interna cells of antral follicles isolated on Day 16 of pregnancy after treatment with a small amount of hCG on Days 14 and 15 of pregnancy. On the other hand, since LH has been shown to increase ovarian blood flow by its ability to release histamine (Szego & Gitin, 1964; Lipner, 1971; Piacsek & Huth, 1971; Niswender *et al.*, 1976; Krishna & Terranova, 1985; Krishna *et al.*, 1986), exogenous LH given during mid-pregnancy in rats may increase ovarian blood flow, thus delivering FSH to the ovary more effectively and increasing the likelihood of follicular maturation.

We conclude that degeneration of large antral follicles during mid-pregnancy in the rat is due to the preceding decline in plasma LH concentrations. Abolishing this reduction in LH activities induced new follicular maturation and/or prevented large antral follicles from degenerating as well as increased ovarian secretion of inhibin and oestradiol-17 β .

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