

Changes in FSH, LH and Prolactin Secretion and Ovarian Follicular Development during Lactation in the Rat

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Abstract

To elucidate the factors regulating ovarian follicular development during lactation in the rat, plasma and pituitary levels of FSH, LH and prolactin (PRL) were determined for each day of lactation along with the histological examination of size-distribution of ovarian follicles.

Post-partum surges in FSH and LH were observed at 1700 hr on Day 0 of lactation (Day 0=day of parturition). Plasma FSH values were within the range of diestrous levels during Days 1 to 21 whereas pituitary content of FSH exceeded the values of 1400 hr of proestrus (the highest level during the estrous cycle) from Day 3 onward. On the other hand, concentrations of plasma LH were generally below the range of diestrous values throughout lactation. From Day 1 onward pituitary content of LH increased slowly but did not attain proestrous levels until Day 14. High concentrations of plasma PRL were observed from Day 4 to 10, although the time of suckling stimulus was not controlled. Pituitary content of PRL had abruptly increased by Day 3. Thereafter, high levels were maintained until Day 17, followed by a sudden decline on day 18.

After post-partum ovulation, the number of healthy follicles larger than 201 μm in diameter abruptly decreased until Day 6. From Day 3 to 7, a few healthy follicles of 201-400 μm were present, but larger follicles were absent. After Day 8, follicles larger than 401 μm were always present.

On the basis of these results it is suggested that during the early stages of lactation, the strong suckling stimulus and the consequent high levels of PRL result in a decreased plasma LH level which is insufficient to cause maturation of the follicles. Ovaries during early stages of lactation, however, were still responsive to exogenous gonadotropins initiating follicular maturation under the influence of normal suckling stimulus and high levels of PRL.

The prolonged surge of follicle-stimulating hormone (FSH) from proestrus to estrus has been postulated as the factor which recruits the follicles destined to ovulate during the next cycle (Schwartz, 1969). This recruitment has been shown in 5-day cyclic rats (Welschen and Dullaart, 1976; Hirshfield and Midgley, 1978) as well as in 4-day cyclic rats (Hoak and Schwartz, 1980) to involve an increase in a new set

of antral follicles. Although the post-partum surge in luteinizing hormone (LH) and FSH normally occurs 3-10 hr after parturition (Rebar *et al.*, 1969; Ying *et al.*, 1973; Morishige *et al.*, 1973; Mori *et al.*, 1974; Labhsetwar and Watson, 1974), no ovulatory response to human chorionic gonadotropin (HCG) is evident until Day 7 of lactation (Day 0=day of parturition), and ovulation is induced by HCG in most of the animals after Day 12 of lactation (Taya and Sasamoto, 1977). On the other hand, ovulation

is readily produced by HCG on any day of pseudopregnancy and in the first half of pregnancy (Taya and Sasamoto, 1977) though serum progesterone levels do not differ among these three types of the anovulatory phase (Pepe and Rothchild, 1974).

The present study was therefore designed to examine the relationship between the follicular population in the ovary and the levels of FSH, LH and prolactin (PRL) in the plasma as well as in the pituitary gland in lactating rats in order to elucidate the factors involved in the follicular development during lactation.

Materials and Methods

Rats of the Wistar strain were housed under lighting conditions of 14 hr light and 10 hr darkness (lights on at 0500 hr). Pregnancy was induced by mating with fertile males during proestrus and pregnant females were placed in separate cages before the expected date of parturition. The day of parturition was designated as Day 0 of lactation and the litter was adjusted to eight pups on Day 1 of lactation. Between 1700 and 1800 hr on each day of lactation, five rats from each group were killed by bleeding from the abdominal aorta, with a heparinized syringe, within 2 min from the time of ether anesthesia. Individual plasma samples were obtained after centrifugation and stored at -20°C until assayed. Anterior pituitary glands which were removed immediately after death were homogenized in 5 ml cold 0.85% (W/V) NaCl solution. After centrifugation, the resulting supernatant fraction was stored at -20°C until assayed. Ovaries were removed, weighed and fixed in Bouin's fluid. The uterus was removed and weighted after pressing out the intraluminal fluid. Serial sections ($10\text{ }\mu\text{m}$) of paraffin-wax-embedded ovaries were stained with hematoxylin and eosin. In each animal, the follicles in one randomly chosen ovary were scanned at a magnification of $50\times$, and all non-atretic follicles larger than $201\text{ }\mu\text{m}$ were measured with an ocular micrometer. The mean diameter is the mean of two perpendicular diameters measured in the section containing the largest cross-section of the oocyte. The diameter measured the limits of the membrana granulosa. The follicles were classified in accordance with the classes listed in Table 1. In our colony of rats, all follicles larger than $301\text{ }\mu\text{m}$ in diameter had a developed antral cavity.

Degenerative phenomena of the oocyte which could be detected by light microscopy, i.e. loss of nucleus or nuclear changes such as pycnosis, change in shape or swelling, were used as criteria for follicular atresia. Changes in the granulosa cells such as pycnosis, shrinkage, luteinization or local thinning of the membrana granulosa were also used as criteria for atresia.

In order to determine the ovarian responsiveness to initiate follicular maturation in mother rats during the early stages of lactation, 10 IU pregnant mare serum gonadotropin (PMS, 1100 IU/mg; provided by Sankyo Zoki Co., Tokyo, Japan) was injected subcutaneously at 0900 hr on Day 3 of lactation. Thereafter, follicular ability to ovulate was examined daily by the i.v. injection of 10 IU human chorionic gonadotropin (HCG, 2200 IU/mg; provided by Sankyo Zoki Co., Tokyo, Japan) as an ovulating hormone. Both PMS and HCG were dissolved in 0.2 ml 0.85% NaCl solution. Ovulation was determined by the presence of tubal oocytes 16–17 hr after the injection of HCG.

Plasma and pituitary levels of FSH, LH and PRL were measured using the NIAMDD rat FSH, LH and PRL radioimmunoassay kits. Antisera used were anti-rat FSH serum-7, anti-rat LH serum-1 and anti-rat PRL serum-2. Preparations for radioiodination were rat FSH I-3, rat LH I-3 and rat PRL I-1. The results were expressed in terms of NIAMDD rat FSH-RP-1, rat LH-RP-1 and rat PRL-RP-1. The intra- and interassay coefficients of variation were 4.10 and 8.75% for FSH, 2.34 and 3.49% for LH and 5.22 and 8.55% for PRL, respectively.

Data were assessed by analysis of variance and Duncan's Multiple Range Test (Steel and Torrie, 1960). Differences were judged as significant if P values were less than 0.05.

Results

Changes in ovarian and uterine weights (Fig. 1)

After parturition, ovarian and uterine weights rapidly declined until Day 7 of lactation, and thereafter both ovarian and uterine weights remained at baseline levels. Post-partum ovulation occurred within 24 hr after parturition with a comparable number of oocytes shed (15.6 ± 1.9 , $n=5$) to cyclic animals.

Follicular development during lactation (Table 1)

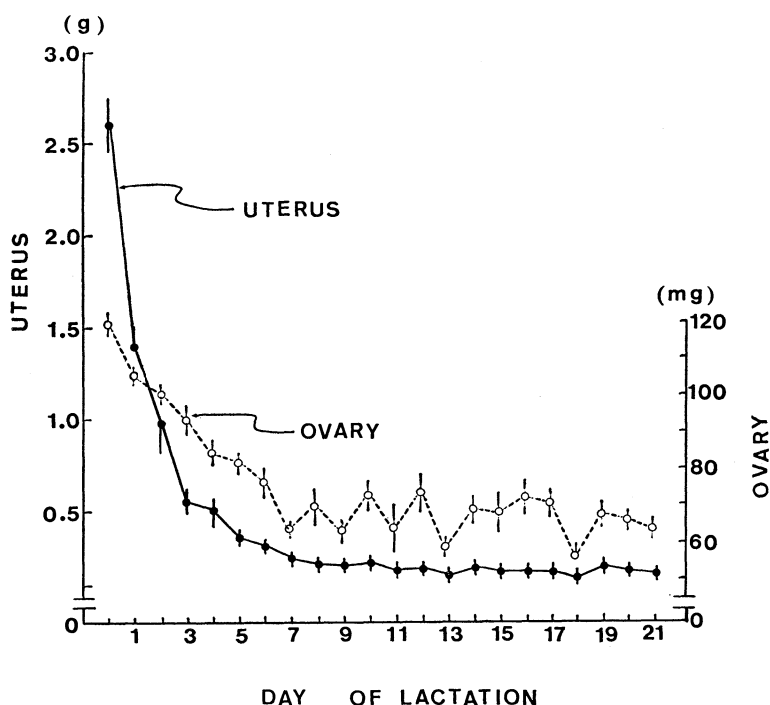


Fig. 1. Changes in ovarian and uterine weights during lactation in the rat. In all figures, the results are the means $\pm$ S.E.M. (vertical lines for 5 animals at each point).

A striking change in ovarian histology was observed in the first half of lactation. On the day of parturition, about 40 follicles larger than $201\ \mu\text{m}$ in diameter per ovary were present. Following post-partum ovulation, the total number of healthy follicles and the mean number of follicles of each size abruptly decreased and reached the nadir by Day 6. Between Days 3 and 7, a few follicles $201\text{--}400\ \mu\text{m}$ in diameter were present, but follicles larger than $401\ \mu\text{m}$ were absent. After Day 8, the mean number of follicles $201\text{--}500\ \mu\text{m}$ in diameter increased, and after Day 12, a small number of follicles $401\text{--}500\ \mu\text{m}$ in diameter were present in all animals. On the other hand, the total number of atretic follicles larger than $201\ \mu\text{m}$ in diameter markedly increased after post-partum ovulation. The maximal number of atretic follicles was found on Days 5 and 6, and then decreased by Day 7. Thereafter, the number of

atretic follicles showed a small fluctuation until Day 21 of lactation.

Follicular responsiveness to PMS during early stages of lactation (Table 2)

To determine the responsiveness of ovarian follicles to gonadotropins during early stages of lactation, 10 IU PMS was injected sc at 0900 hr on Day 3 and the subsequent maturity of the antral follicles was examined by noting the ability of follicles to ovulate after 10 IU HCG. When HCG was given at 1700 hr on Day 4 (32 hr later), all animals ovulated the next morning with an average of 10.3 oocytes. The administration of HCG at 1700 hr on Day 5, 6 or 7 also produced ovulation the next morning in all animals with no further increase in the number of oocytes shed. After the administration of PMS on Day 3 without further HCG treatment, none ovulated until Day 8 and vaginal smears

Table 1. Follicle size and number per ovary during

Size of follicles (μm)	Day 0*	Day 1	Day 2	Day 3	Mean No. Day 4
201-300	21.0 $\pm$ 3.1(5)	7.3 $\pm$ 0.8(4)	10.6 $\pm$ 1.3(5)	6.6 $\pm$ 0.8(5)	4.2 $\pm$ 0.7(5)
301-400	9.8 $\pm$ 1.7(5)	12.0 $\pm$ 2.0(4)	6.6 $\pm$ 0.5(5)	2.3 $\pm$ 0.9(4)	3.0 $\pm$ 1.0(3)
401-500	3.0 $\pm$ 0.7(4)	6.0 $\pm$ 1.8(4)	3.4 $\pm$ 0.4(5)	—	—
501-600	3.2 $\pm$ 0.7(5)	2.0 $\pm$ 0.6(3)	1(1)	—	—
601	4.2 $\pm$ 0.6(5)	—	—	—	—
Total $\geq$ 201	40.6 $\pm$ 3.8(5)	26.8 $\pm$ 1.4(4)	20.8 $\pm$ 1.6(5)	8.6 $\pm$ 1.1(5)	6.0 $\pm$ 1.1(5)
Atretic follicles $\geq$ 201	11.6 $\pm$ 1.8(5)	19.3 $\pm$ 1.8(4)	40.6 $\pm$ 2.3(5)	39.8 $\pm$ 6.4(5)	39.0 $\pm$ 6.3(5)
Size of follicles (μm)	Day 11	Day 12	Day 13	Day 14	Day 15
201-300	6.0 $\pm$ 1.4(4)	7.2 $\pm$ 1.8(5)	8.2 $\pm$ 2.4(5)	6.2 $\pm$ 1.2(5)	8.2 $\pm$ 1.1(5)
301-400	2.3 $\pm$ 0.8(4)	7.8 $\pm$ 2.1(5)	4.8 $\pm$ 1.1(5)	5.0 $\pm$ 1.1(5)	4.3 $\pm$ 0.9(4)
401-500	1.5(2)	3.4 $\pm$ 1.7(5)	2.3 $\pm$ 0.9(4)	3.4 $\pm$ 0.4(5)	2.0 $\pm$ 0.4(5)
501-600	1(1)	1(1)	—	1.3 $\pm$ 0.3(3)	1.0 $\pm$ 0.0(3)
601	—	—	—	—	—
Total $\geq$ 201	9.3 $\pm$ 1.0(4)	18.6 $\pm$ 4.7(5)	14.8 $\pm$ 2.8(5)	15.6 $\pm$ 1.5(5)	14.2 $\pm$ 2.0(5)
Atretic follicles $\geq$ 201	31.8 $\pm$ 3.9(4)	30.2 $\pm$ 4.5(5)	24.6 $\pm$ 3.1(5)	22.8 $\pm$ 2.5(5)	21.0 $\pm$ 2.9(5)

The number in parentheses indicates the number of animals having follicles in each size range.

* Day 0=day of parturition.

of the diestrous type continued though ovarian follicles developed to the stage capable of ovulating.

Plasma concentrations of FSH, LH and prolactin (Figs. 2 and 3)

A striking increase in plasma levels of FSH and LH was observed at 1700 hr on Day 0 of lactation, since all of animals in this group completed parturition on the morning of the same day (Fig. 2). Plasma concentrations of FSH remained lower from Day 4 to 9 than the diestrous level with no statistical difference. Thereafter, plasma FSH levels were maintained at basal levels throughout the period of lactation comparable to diestrous levels in the estrous cycle. On the other hand, plasma LH concentrations in lactating rats were constantly lower than those of diestrous animals during the estrous cycle.

Healthy antral follicles larger than 401 μm in diameter were absent from Day 3 to 7, and antral follicles in this size range reappeared in the ovary after Day 8 and further increased thereafter. Plasma levels

of FSH measured three times per day during Days 3 to 12 showed no significant increase at any time throughout the period of observation, though there were large variations within the groups (Fig. 3).

There was a marked increase in plasma levels of PRL on Day 4 and high levels were maintained until Day 10. Thereafter, the plasma PRL was relatively low until the end of lactation. Concentrations of plasma PRL, however, showed large variations on almost every day of lactation, since the mothers were not separated from their pups before killing.

Pituitary contents of FSH, LH and prolactin (Fig. 4)

In our colony of rats during the estrous cycle, the maximum FSH and LH content in the pituitary gland was observed at 1400 hr on the day of proestrus, just before the preovulatory FSH and LH surge. Pituitary FSH and LH content was low at 1700 hr on Day 0 when post-partum FSH and LH surges were in progress. Pituitary FSH content started to increase on Day 1, rea-

lactation in the rat. (Values are mean $\pm$ S.E.M.)

follicles					
Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
3.8 $\pm$ 1.4(4)	3.0 $\pm$ 1.0(4)	4.0 $\pm$ 1.4(4)	6.6 $\pm$ 2.0(5)	6.2 $\pm$ 1.8(5)	3.8 $\pm$ 0.7(5)
1.7 $\pm$ 0.7(3)	2.0 $\pm$ 0.6(3)	2.3 $\pm$ 0.7(4)	5.0 $\pm$ 0.8(5)	3.3 $\pm$ 0.6(4)	3.5 $\pm$ 1.0(4)
—	—	—	2.6 $\pm$ 0.6(5)	2.3 $\pm$ 0.9(3)	4.5 $\pm$ 1.3(4)
—	—	—	—	1.5(2)	1(1)
—	—	—	—	—	—
5.0 $\pm$ 1.5(5)	3.8 $\pm$ 1.0(4)	6.3 $\pm$ 1.5(4)	14.2 $\pm$ 2.3(5)	10.8 $\pm$ 1.2(5)	10.4 $\pm$ 0.8(5)
52.0 $\pm$ 5.4(4)	48.5 $\pm$ 8.5(4)	30.3 $\pm$ 3.4(4)	29.8 $\pm$ 1.6(5)	24.0 $\pm$ 1.7(5)	30.8 $\pm$ 3.1(5)
Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
7.8 $\pm$ 0.5(5)	6.8 $\pm$ 1.7(5)	7.6 $\pm$ 1.5(5)	8.4 $\pm$ 1.2(5)	6.2 $\pm$ 1.7(5)	6.4 $\pm$ 1.3(5)
5.8 $\pm$ 1.2(5)	3.2 $\pm$ 0.4(5)	5.1 $\pm$ 1.3(5)	7.0 $\pm$ 1.9(5)	3.4 $\pm$ 0.8(5)	2.8 $\pm$ 0.6(5)
1.6 $\pm$ 0.4(5)	3.0 $\pm$ 0.9(5)	3.0 $\pm$ 0.9(5)	2.6 $\pm$ 0.4(5)	3.8 $\pm$ 1.3(4)	2.2 $\pm$ 0.5(5)
1.5(2)	1.5(2)	1(2)	1.5(2)	1.0 $\pm$ 0.0(5)	1(1)
—	—	—	—	—	—
15.8 $\pm$ 1.0(5)	13.6 $\pm$ 2.8(5)	14.2 $\pm$ 3.1(5)	18.6 $\pm$ 3.5(5)	13.6 $\pm$ 2.1(5)	11.6 $\pm$ 1.2(5)
30.2 $\pm$ 3.0(5)	29.8 $\pm$ 2.9(5)	18.0 $\pm$ 2.4(5)	18.4 $\pm$ 2.5(5)	25.4 $\pm$ 1.3(5)	21.0 $\pm$ 0.5(5)

ching values exceeding proestrous levels on Day 3. A further increase was observed by Day 7 and these high levels of pituitary FSH were maintained until the end of lactation. In contrast, pituitary content of LH gradually increased, but the values remained lower until Day 13 than the proestrous level. On Day 14, pituitary LH reached the proestrous level and remained so until the end of lactation.

An abrupt increase in pituitary PRL content was noted from Day 0 to 3. A further increase was observed by Day 9 and these high levels were maintained until Day 17, followed by a marked decline on Day 18. Thereafter, pituitary PRL content was comparable to the proestrous level and was maintained until the end of lactation.

Discussion

The present experiment suggest that the FSH surge during post-partum ovulation causes the appearance of a set of antral follicles (301–500 μ m in diameter) on Day

Table 2. Induction of ovulation by 10 IU HCG (i.v.) given at various times after the administration of 10 IU PMS (s.c.) at 0900 hr on Day 3 of lactation (Values are mean $\pm$ S.E.M.). Animals were killed the next morning after HCG injection to examine the oviducts for oocytes.

Time of HCG	Treatment on Day 3	rats ovulating/ rats examined	No. of oocytes in ovulating rat
1700 hr on Day 4	PMS	4/4	10.3 $\pm$ 1.0
	None	0/5	0
1700 hr on Day 5	PMS	4/4	8.3 $\pm$ 1.0
	None	0/5	0
1700 hr on Day 6	PMS	4/4	11.0 $\pm$ 1.9
	None	0/5	0
1700 hr on Day 7	PMS	4/4	6.0 $\pm$ 2.2
	None	0/5	0

1 of lactation. However, after Day 2 of lactation, no further development and maintenance of antral follicles took place. During Days 3 to 7, the total number of healthy follicles larger than 201 μ m and the mean number of follicles 201–400 μ m in

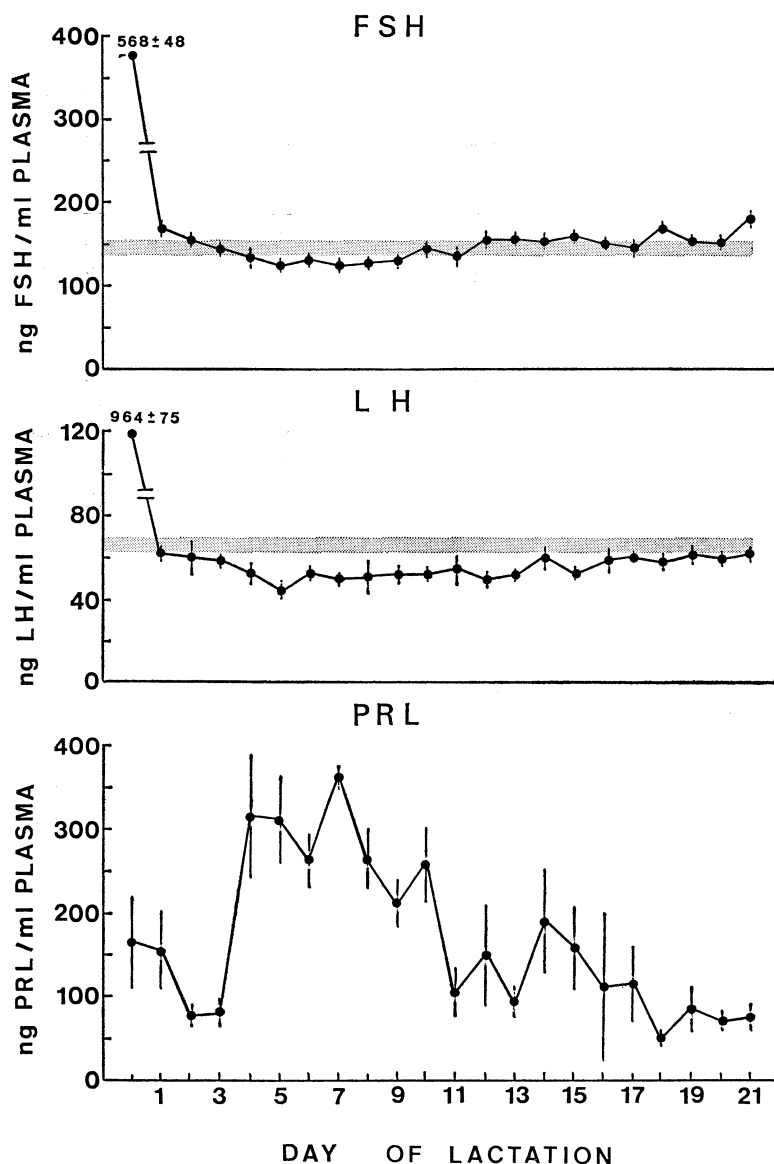


Fig. 2. Plasma levels of FSH, LH and PRL during lactation in the rat. The shaded portion of the graph represents the means $\pm$ S.E.M. of plasma FSH and LH levels at 1700 hr on diestrus II of cyclic rats.

diameter decreased, and no follicles larger than $401\ \mu\text{m}$ in diameter were present (Table 1). On the other hand, the total number of atretic follicles increased during this period, indicating that all of the follicles recruited by the post-partum FSH surge became atretic after Day 2 of lactation. The present data also confirmed our previous

observations that no ovulation in response to HCG occurred until Day 7 of lactation (Taya and Sasamoto, 1977). However, the ovaries are still capable of initiating follicular maturation during the early stages of lactation by the administration of gonadotropins (Table 2). After Day 8 of lactation, large healthy follicles ($401\text{--}600\ \mu\text{m}$) were

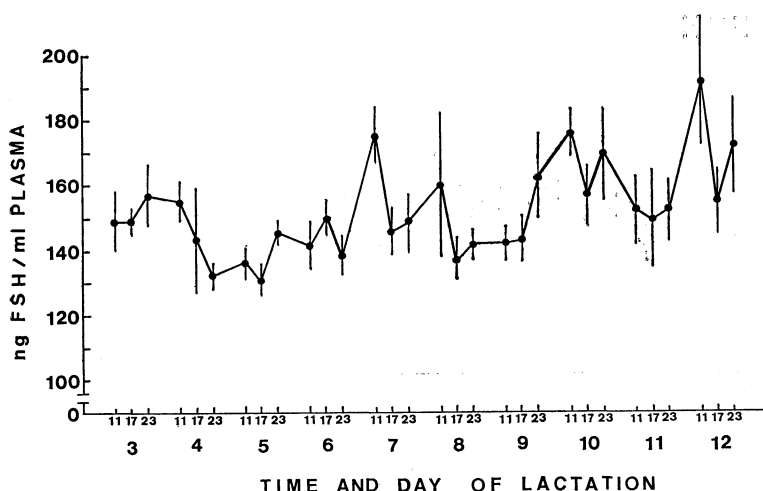


Fig. 3. Plasma levels of FSH during Days 3 to 12 of lactation in the rat.

always present (Table 1). An increase in the ovulatory response to HCG occurs after Day 8 of lactation and the follicles are highly responsive to HCG after Day 12 of lactation (Taya and Sasamoto, 1977).

In the mouse, Greenwald (1958) has demonstrated that during the first 11 days post-partum, the largest vesicular follicles were 350 μ m in diameter. However, significant changes occurred after 12 days post-partum: the size of the follicles in most animals increased to 400–450 μ m in diameter. Our present results on follicular population in the ovary of the lactating rat were similar to those of the lactating mouse.

A large amount of FSH released from the afternoon of proestrus to the morning of estrus in the cyclic rat is presumably responsible for the initiation of follicular maturation for the succeeding estrous cycle (Welschen and Dullaart, 1976; Hirshfield and Midgley, 1978; Hoak and Schwartz, 1980). When the litter is removed on Day 3 of lactation, spontaneous ovulation occurs 4 days later. A small surge in FSH takes place within 36 hr and is assumed to be responsible for the initiation of follicular maturation after removal of the litter (Taya and Sasamoto, 1980). No obvious surge in

plasma FSH was observed during Days 3 to 12 of lactation (Figs. 2 and 3) though a small but non significant increase was noted on Days 7, 10 and 12. Whether these small changes in plasma FSH may play a role in follicular development or whether FSH may not be involved in the initiation of follicular maturation during the first half of lactation has not been determined by the present experiments.

Recently, Richards and Kersey (1979) reported that follicles ovulating immediately post-partum develop in the absence of the FSH surge during pregnancy. They have suggested that the development of preovulatory follicles does not necessarily require the surge levels of FSH observed in the estrous cycle. Rather, follicular cell differentiation can proceed in response to more subtle increases in serum levels of gonadotropins and declining levels of serum progesterone. Similar mechanisms may be involved in follicular development in the first half of lactation in the rat probably due to a declining of ovarian progesterone secretion after Day 8 of lactation (Tomogane *et al.*, 1969).

Concentrations of plasma LH were below the range of those at diestrus in the estrous cycle throughout the period of lactation

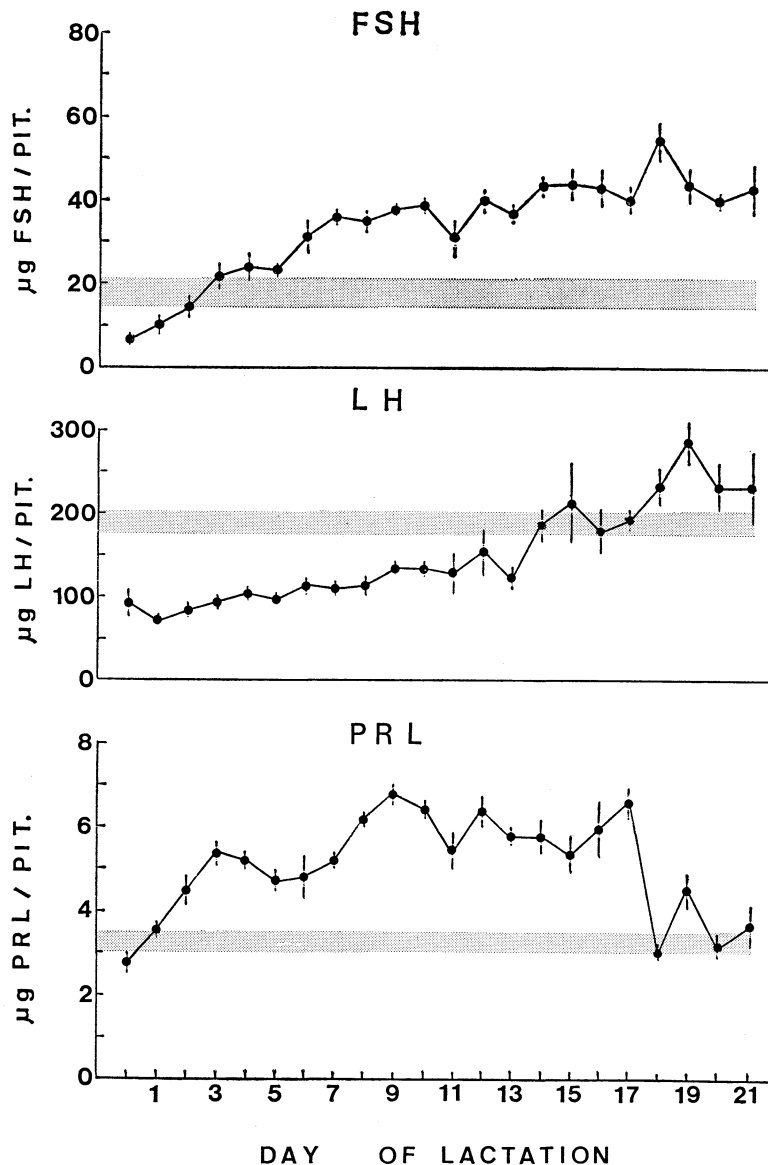


Fig. 4. Pituitary levels of FSH, LH and PRL during lactation in the rat. The shaded portion of the graph represents the means $\pm$ S.E.M. of pituitary FSH, LH and PRL levels at 1400 hr on proestrus of cyclic rat.

(Fig. 2). Pituitary LH content was also below the range of those at proestrus until Day 14 of lactation (Fig. 4). As LH plays an important role in follicular steroidogenesis in the rat (Dorrington *et al.*, 1975; Moon *et al.*, 1975; Armstrong and Papkoff 1976; Erickson and Hsueh, 1978; Uilenbroek and Richards, 1979; Richards and Kersey, 1979), the low levels of plasma LH

during the first half of lactation may be insufficient to support thecal cell function and androgen biosynthesis, thereby resulting in suppression of estrogen secretion by granulosa cells. Thus no follicular maturation may take place. There is significantly less LH-RH in the hypothalamus and less LH content in the pituitary of lactating rats than non-lactating female rats (Minaguchi

and Meites, 1967; Smith and Neill, 1977; Smith, 1978a). In the lactating rat, the degree of suppression of LH is directly related to the intensity of the sucking stimulus (Ford and Melampy, 1973; Hammons *et al.*, 1973).

High levels of plasma PRL were observed in our studies during Days 4 to 10, with large variations in each group (Fig. 2). A high pituitary PRL content was also observed during Days 3 to 17, indicating that both release and synthesis of PRL were accelerated during early lactation (Fig. 4). Smith (1978b) reported that the suckling stimulus contributes more than PRL to the suppression of the postcastration rise in LH during early lactation whereas during the later part of lactation, as the suppressive effects of the suckling stimulus wane, PRL alone accounts for the decrease in LH and FSH secretion. Murlidhar *et al.* (1977) found that exogenous PRL, in the presence of a minimum suckling stimulus, decreased pituitary responsiveness to LH-RH. The antigonadotropic effects of PRL have also been demonstrated under other conditions. High levels of serum PRL induced by pituitary transplants result in suppression of the typical pulsatile LH release observed in ovariectomized female rats and of basal LH levels in immature female rats and adult male rats and mice (Beck and Wuttke 1977; Beck *et al.*, 1977; Bartke *et al.*, 1977).

It has been reported that a large amount of progesterone is secreted from the functional corpora lutea during the first half of lactation in the rat (Tomogane *et al.*, 1969; Yoshinaga *et al.*, 1971; Pepe and Rothchild, 1974; Smith and Neill, 1977). High levels of plasma progesterone may suppress follicular maturation indirectly, most probably by lowering serum LH. Exogenous progesterone injected into diestrous rats lowered serum levels of LH within 1 hr (Taya and Greenwald, 1981). Further priming with progesterone diminished ^3H -thymidine uptake by small preantral follicles stimulated

by FSH in hypophysectomized hamster (Chiras and Greenwald, 1978), suggesting a direct inhibitory effect of progesterone on follicular development.

In conclusion, the results of our studies in conjunction with previous reports suggest that during the period of early lactation, plasma levels of LH are insufficient to support the initiation of follicular maturation. In addition, active corpora lutea, probably via progesterone, may directly affect follicular development within the ovary.

Acknowledgements

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