Plasma Levels of Prolactin, FSH and LH in the Pseudopregnant Rat[1] (36777)

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The mechanism of control of the mammalian corpus luteum is not well understood and is currently under active investigation. The functional life span of the corpora lutea in the normal cycling rat is very short. It is well known that the corpora lutea may be maintained for long periods after mechanical stimulation of the cervix (1), electrical stimulation of the cervix (2) and by injection of various hormones (3–5) or drugs (6). This extended period of luteal function has great similarity to the luteal state of pregnancy.

The luteotropic influence of prolactin in the rat has been known for a long time (7, 8) although the details of its involvement in the luteal phase are not yet clear. Increasing amounts of information indicate the possible involvement of other pituitary hormones in the control of the luteal phase in various species (9). Nonpituitary hormones are also involved, either directly or indirectly in this process: estrogen is now accepted as luteotropic in the rabbit (10).

The purpose of this experiment was to determine the secretory patterns for prolactin, FSH and LH throughout the duration of pseudopregnancy.

Materials and Methods. Virgin female rats of the Sprague-Dawley Strain (Simonsen, Gilroy, CA) weighing 175–200 g were housed, two per cage, in light (5 PM–7 PM) and temperature-controlled rooms. They were maintained on Purina Rat Chow and water *ad libitum*. Only rats showing two consecutive estrous cycles of either 4 or 5 days duration were used.

Pseudopregnancy was induced by mechanical stimulation of the cervix on the morning of estrus; each rat was stimulated for 60 sec with a vibrator (11). The day of stimulation was designated as Day 0 and the average duration of pseudopregnancy obtained by this method was 12 ± 1 days.

All rats were decapitated at 5 PM on designated days and stages of the estrous cycle. Between 4 and 13 rats were sacrificed at each time interval. The blood was heparinized and the plasma was separated by centrifugation and maintained frozen at —20° until assayed. All three hormones were measured by double antibody radioimmunoassay using the NIAMD reagents. The values obtained are expressed in terms of the RP-1 reference standard. Significance of differences between values obtained at the various times in pseudopregnancy were determined by calculation of F values utilizing analysis of variance and student t tests (12).

Results. Measurements of FSH, LH and prolactin during the estrous cycle indicated that all three hormones reached a peak on the afternoon of proestrus (Table I). No significant differences were observed among the lower values measured for each hormone during the remaining days of the cycle.

Plasma levels of prolactin (Fig. 1) on Day 0 of pseudopregnancy (203 ± 56 ng/ml) were significantly higher ($p < .001$) than values obtained in the afternoon of estrus. No significant differences exist between values observed on Days 1–5. On Day 6, a sharp decrease in the plasma prolactin was observed which did not change on the two fol-

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TABLE I. Plasma Levels of Hormones During the Estrous Cycle.

	Prolactin (ng/ml)	LH (ng/ml)	FSH (ng/ml)
Proestrous	108 ± 8[a]	710 ± 99	805 ± 74
Estrus	22 ± 5	83 ± 4	378 ± 21
Diestrus I	16 ± 8	84 ± 7	362 ± 28
Diestrus II	15 ± 4	77 ± 7	321 ± 23

[a] Mean ± SE.

lowing days. On Day 9, however, the prolactin levels were significantly ($p < .05$) higher than Day 8, but still lower than Day 4 of pseudopregnancy ($p < .025$). On Days 10 and 11, prolactin values were not significantly different from those on Days 9 and 8 of pseudopregnancy.

FSH levels during pseudopregnancy were all significantly lower ($p < .001$) than values obtained for animals in proestrus. The FSH profile (Fig. 2) showed a peak on Day 3 of pseudopregnancy which was significantly higher than Day 2 ($p < .01$). FSH values declined progressively between Day 3 and 6. A steady rise in FSH was observed on Day 8 and maintained throughout the remaining days of pseudopregnancy with values on Day 11 being significantly different from Day 8 ($p < .02$).

The relatively uniform plasma levels of LH (Fig. 3) throughout pseudopregnancy were all significantly lower ($p < .001$) than values obtained for animals in proestrus. Hormonal levels during pseudopregnancy in rats which had previously exhibited 4 day estrous cycles did not differ from those that had 5 day cycles.

Discussion. Ample evidence exists for the involvement of a neurogenic pathway in the induction of pseudopregnancy in the female rat. Long and Evans in 1922 (13) were able to induce pseudopregnancy in the rat by mechanical stimulation of the cervix uteri. The nature of the hormonal changes occurring in response to the cervical stimulation and subsequent events which lead to the maintenance of pseudopregnancy have been of great interest. Section of the pelvic nerves prior to cervical stimulation blocks the rise in plasma prolactin and subsequent induction of pseudopregnancy (14). To our knowledge, extensive and direct measurements of the plasma values of prolactin and the gonadotropins throughout pseudopregnancy have not been made. However, these hormones have been quantified for a limited number of days during pseudopregnancy (15, 16).

The presumed role of prolactin is the maintenance of progesterone secretion by the rat corpora lutea. There is convincing evidence that prolactin does so by antagonizing the enzyme 20α-OH-steroid dehydrogenase and thus prevents the conversion of progesterone to its nonfunctional metabolite

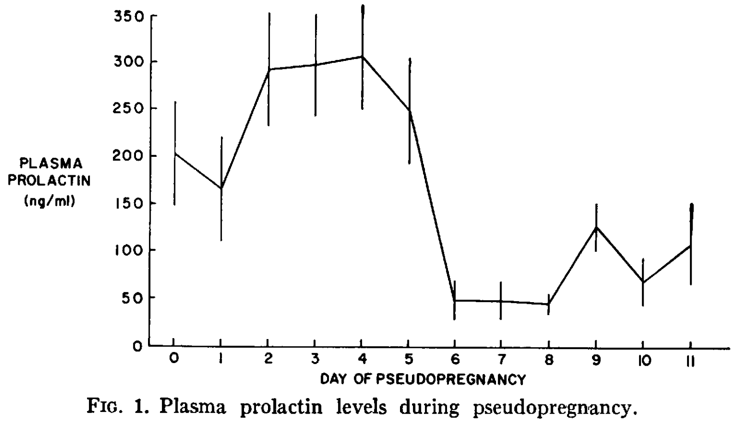

FIG. 1. Plasma prolactin levels during pseudopregnancy.

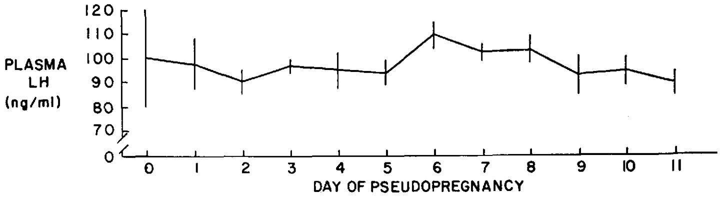

FIG. 2. Plasma LH levels during pseudopregnancy.

20α-OH-progesterone (17). Moreover, some evidence exists for the involvement of prolactin in the mobilization of precursors for progesterone biosynthesis (17). However, it is not known whether the prolonged progesterone production is dependent upon prolonged elevation of prolactin levels. The results observed for prolactin in this study correlate closely with progesterone profile observed during pseudopregnancy reported by Hashimoto and Wiest (17). The progesterone levels in their study were high over the first 5 days with a peak observed on Day 4 of pseudopregnancy. The second prolactin peak observed on Day 9 of pseudopregnancy could not be correlated with any known physiological event, and may reflect animal-to-animal variation.

Many theories have been set forth regarding the involvement of LH in the control of the luteal phase. *In vitro* studies using rat ovaries have shown that LH promotes steroidogenesis (18). Some investigators have suggested LH as being the primary luteotropin in the rat (9). However, the results of this study indicate that no prolonged elevated levels of LH are present to support such a concept. Although these (9, 18) and other studies (19, 20) indicate the possible involvement of other hormones in addition to prolactin in the maintenance of progesterone secretion, our results suggest that prolactin is the critical hormone in the luteotropic process of the rat. The low level of LH maintained throughout pseudopregnancy could have a synergistic effect on the luteotropic role of prolactin; however, the striking feature of the LH profile is its low position which is compatible with the anovulatory state of pseudopregnancy.

The role of FSH, if any, in luteal activation and maintenance is unknown. There is evidence for the existence of growing follicles in the ovaries of pseudopregnant rats (21). The concept that slowly growing follicles need to be under the stimulatory influence of FSH would fit our data. At present, we cannot offer a satisfactory explanation for the plasma elevation of FSH on the third day of pseudopregnancy. Nevertheless, a parallel observation has been made in both the rat and the mouse (22). The pituitary content of FSH, assayed biologically by its ability to stimulate uterine growth, was observed to decline on the third day of pseudopregnancy. The data were interpreted as a reflection of FSH release into the plasma on this day. The steady rise observed beginning Day 8 of pseudopregnancy could account for the rapid follicular development indicative of preovula-

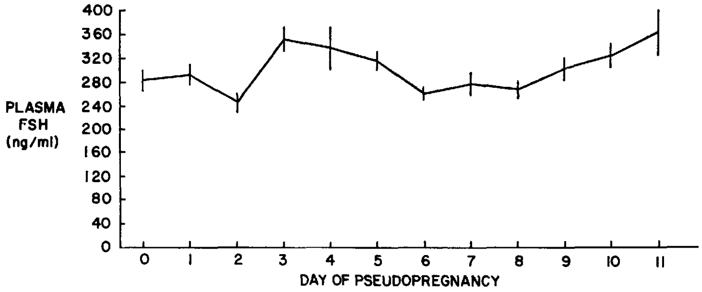

FIG. 3. Plasma FSH levels during pseudopregnancy.

tory follicles.

Summary. The present study provides a comprehensive assessment of the dynamic changes that occur in the secretion of prolactin, LH and FSH during pseudopregnancy. Prolactin levels are elevated during the first 5 days of pseudopregnancy and suppressed during the remainder of the term. LH levels are suppressed during the duration of pseudopregnancy and show very little fluctuation. Finally, FSH levels, while lower than values of this hormone in proestrus, show a peak on Day 3 and a steady rise beginning Day 8 of pseudopregnancy.

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1. De Feo, V. J., Endocrinology **72**, 305 (1963).
2. Staples, R. E., Anat. Rec. **152**, 499 (1965).
3. Alloiteau, J. J., C. R. Soc. Biol. **151**, 290 (1957).
4. Everett, J. W., Nature (London) **198**, 695 (1963).
5. Alloiteau, J. J., and Vignal, A, C. R. Acad. Sci. **247**, 2465 (1958).
6. Barraclough, C., and Sawyer, C. H., Endocrinology **65**, 563 (1959).
7. Astwood, E. B., Endocrinology **28**, 309 (1941).
8. Evans, L. M., Simpson, M. E., Lyons, W. R., and Turpeinen, K., Endocrinology **28**, 933 (1941).
9. Madhwa Raj, H. G., and Moudagal, N. R., Endocrinology **86**, 874 (1970).
10. Fuller, G. B., and Hansel, W., Proc. Soc. Exp. Biol. Med. **137**, 539 (1971).
11. De Feo, V. J., Endocrinology **79**, 440 (1966).
12. Snedecor, G. W., and Cochran, W. G., "Statistical Methods," 6th ed. Iowa State Univ. Press, Ames (1967).
13. Long, J. A., and Evans, H. M., Mem. Univ. Calif. **6**, 1 (1922).
14. Spies, H. G., and Niswinder, G., Endocrinology **88**, 937 (1971).
15. Bishop, W., Orias, R., Fawcett, C. P., Krulich, L., and McCann, S. M., Proc. Soc. Exp. Biol. Med. **137**, 1411 (1971).
16. Deis, R. P., and Alonso, N., Proc. Int. Union Physiol. Sci., **9** (Int. Congr., 25th Munich, 1971), p. 390.
17. Hashimoto, I., and Wiest, W. G., Endocrinology **84**, 873 (1969).
18. Armstrong, D. T., O'Brien, J., and Greep, R. O., Endocrinology **75**, 488 (1964).
19. Abubakar, A., Yoshinaga, S., and Yoshinaga, K., Biol. Reprod. **3**, 31 (1970).
20. Armstrong, D. T., Progr. Endocrinol. Excerpta Med. Found. Int. Congr. Ser. **184**, 89 (1968).
21. Van Rees, G. P., Reproduction in the Female Mammal, 177 (1966).
22. Bindom, B. N., J. Endocrinol. **44**, 349 (1969).

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