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Progesterone Levels Measured Every Two Hours in the Cyclic Hamster (38733)

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Using the competitive protein binding assay, we have previously determined plasma concentrations of progesterone at selected times during the hamster estrous cycle (1, 2). The availability of a more sensitive and specific radioimmunoassay (RIA) now makes it feasible to determine progesterone levels at frequent intervals from the same animal. The present study therefore provides a more comprehensive view of the peripheral pattern of progesterone by sequential sampling of blood from unanesthetized hamsters during the 4-day cycle.

Materials and Methods. Female Syrian Hamsters (*Mesocricetus auratus*), weighing 60–100 g and maintained on a 14-hr light: 10-hr dark schedule (lights on 0500; C.S.T.) were used after three consecutive 4-day cycles. Day 1 of the cycle was the day of ovulation, characterized by a conspicuous vaginal discharge; day 4 corresponds to proestrus.

Four groups of six unanesthetized animals each were bled via cardiac puncture three times on *each* day of one cycle, beginning on day 1, at the following hours: *Group 1:* 0600, 1000, 1400 hr; *Group 2:* 0800, 1200, 1600 hr; *Group 3:* 1800, 2200, 0200 hr; *Group 4:* 2000, 2400, 0400 hr.

On day 1 of the next cycle, all hamsters were killed between 0900–1000 hr. The distinctive new corpora lutea were counted and the oviducts were flushed to confirm ovulation. Of the 24 hamsters constituting this study, 22 ovulated an average of 9.6 ± .40 eggs (±SE); the eggs were still embedded in granulosa cells. The remaining two animals had not ovulated by day 1 but the large, bulging follicles indicated that ovulation was imminent. The progesterone values of the latter two animals were included in the group averages.

Collected blood (0.1 ml) was allowed to clot at 4°; the serum was withdrawn and then stored at −12° until assayed for progesterone by a previously validated radioimmunoassay (3). Anti-progesterone serum (IHT-R-11-1516-1) was provided by Dr. Ian Thorneycroft (4). Where appropriate the data were analyzed by the Student's *t* test.

Results and Discussion. The results are summarized in Fig. 1. Of the five peaks of serum progesterone observed on day 1 of the cycle, only the third one (at 1600 hr) was significantly different from the low values on either side of the peak ($P < 0.01$). These fluctuations, although striking, may not be of physiological consequence as gonadotropins and prolactin on day 1 of the cycle (5) do not fluctuate in a pattern that could account for the waxing and waning in progesterone secretion. It is possible that the excursions on day 1 reflect random animal variation; the first three peaks represent progesterone levels of animals in Group 1, while the remainder were found in hamsters of Group 4.

Progesterone levels were fairly steady on day 2 and were maintained until 0200 hr of day 3. Based on *in vivo* (1) and *in vitro* (6) determinations of luteal progesterone, the abrupt decline in serum levels of progesterone between 0200 and 1000 hr on day 3 represents the functional demise of the corpora lutea. From the lowpoint of progesterone on day 3, a significant increase ($P < 0.05$) occurred by 1200 hr with a still more appreciable increase by 1600 hr ($P < 0.01$). Progesterone on the afternoon of day 3 presumably arises from a nonluteal source and large antral follicles undergoing atresia at this stage of the cycle (7) may be the source of the hormone.

On day 4 (proestrus) progesterone levels began to increase at 1400 hr but was not significantly elevated until 1600 hr. The increase in progesterone at 1400 hr cor-

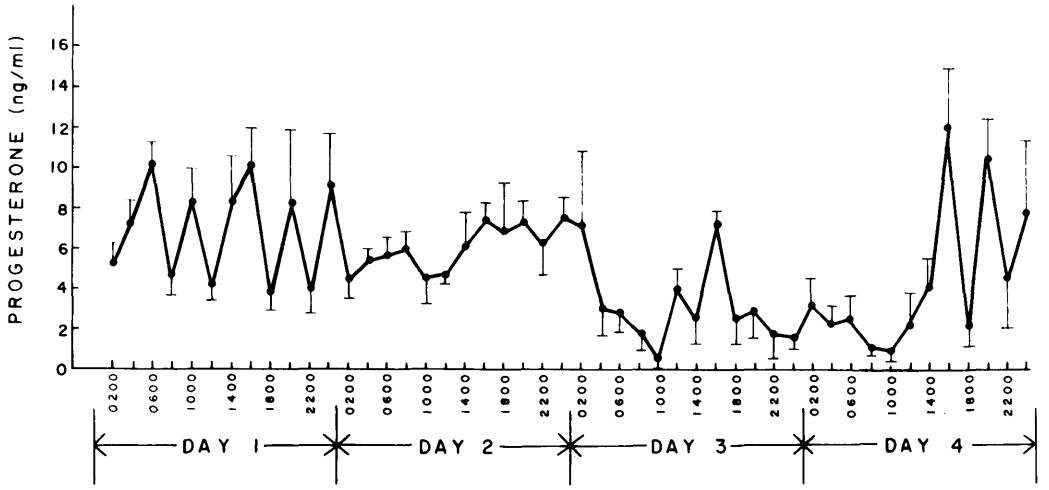

FIG. 1. A composite picture of plasma progesterone (ng/ml + SE) during the estrous cycle of the hamster based on serially bleeding four groups of six unanesthetized animals each three times a day at 4-hr intervals.

relates with the beginning of the LH surge at 1300 (4) which initiates progesterone secretion in the hamster by the antral follicles and especially by the interstititium (2). It is therefore unlikely that the hamster adrenal contributes appreciable progesterone to facilitate LH release, as postulated for the rat (8).

After the initial peak of progesterone on day 4 the levels subsided at 1800 hr ($P < 0.05$). This was followed by a series of cyclic fluctuations comparable to the trend observed on day 1. The progesterone peak at 2000 hr of day 4 was significantly different from the value at 1800 hr ($P < 0.01$).

The fluctuations observed on the afternoon of day 4 were unexpected since previous studies of the hamster (2, 9) showed a sustained increase in progesterone from 1800 hr until at least 2400 hr. These studies measured progesterone by competitive protein binding (2) or gas liquid chromatography (9) in animals killed at selected times on day 4. The cyclic pattern of progesterone shown on day 4 in the present study may result from the use of a different assay technique (RIA) or from random animal variation.

It seems unlikely that the episodic pattern of progesterone secretion on days 1 and 4 represents a stress induced release of adrenal progesterone since the same animals failed to show this pattern when bled on the other days of the cycle. Moreover, the same values of progesterone are observed when hamsters are bled by cardiac puncture or by decapitation (Greenwald, unpublished observations).

Episodic secretion of progesterone occurs in the human during the follicular phase of the cycle and the fact that the peaks of progesterone are not in phase with corticoid secretions suggests an ovarian origin for most of the progesterone (10). This may also be true of the hamster because ovarian concentrations of progesterone are elevated on days 1 and 4 correlating respectively with luteal and nonluteal origin of the hormone (1, 2).

Summary. Four groups of unanesthetized hamsters were bled by cardiac puncture three times a day at 4-hr intervals for each day of the 4-day estrous cycle. Serum progesterone (P) was determined by RIA. On day 1 of the cycle (day of ovulation) there was a trend for excursions in P at 4- to 6-hr intervals; this was followed on day 2 by a relatively steady level of P of 6–8 ng/ml. P dropped drastically after 0200 of day 3, indicating the onset of luteolysis. A significant increase in P occurred at 1600 of day 3 which was presumably nonluteal in origin. A series of cyclic fluctuations in P began at 1600 of day 4 (proestrus) comparable to the pattern observed on day 1 of the cycle.

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