Effect of a Intrauterine Foreign Body During the Estrous Cycle on Plasma Progesterone in Guinea Pigs[1] (35854)

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It has been reported that glass beads inserted into the uterus of the guinea pig shortened the length of the estrous cycle and trauterine foreign body (IUFB) than on CL (1). A reduction in cycle length has also been reported when a plastic tube was inserted into the uterus (2). In both reports, there was a greater effect on CL which were adjacent to a uterine horn containing an intrauterine foreign body (IUFB) than on CL in the opposite ovary. Cycle length is also reduced in cattle (3) and sheep (4) when an IUFB is present. Of interest would be the effect of the IUFB in the guinea pig on the steroidogenic function of the CL. The present experiment was designed to determine peripheral plasma progesterone concentrations during the normal estrous cycle and during the cycle in which an IUFB was present.

Materials and Methods. Animals. Twenty adult (500–800 g) virgin female guinea pigs were used. They were maintained on guinea pig chow (Purina Laboratories) and housed four to five per cage. The occurrence of regular estrous cycles (15–18 days) was determined before and during the experiment by daily observations for vaginal opening. The day the vagina was fully open was designated as day 0 of the cycle. The IUFB were inserted on day 2 or 3 of the cycle. Bleeding was not begun until the beginning of the next cycle.

Surgery and blood collection. The IUFB was sterile plastic tubing (Intramedic polyethylene, PE 280, Clay-Adams, Inc., New York) that was 7.0 mm long and 3.3 mm in diameter. Feed was withheld for 12 hr prior to surgery. Anesthesia consisted of intraperitoneal injection of pentobarbital sodium (40 mg/kg of body wt) supplemented with ether. The ovaries and uterus were exposed by one incision through the lower abdomen. After a small incision of the caudal portion of both uterine horns, the IUFB were inserted and sutured in place in this region with one piece of sterile silk thread. The incision was closed with 5-0 catgut.

Blood was collected from the left ventricle under light anesthesia (pentobarbital sodium, 30 mg/kg). Heparinized 5-ml glass syringes and 20-gauge needles were used for blood withdrawal from the heart. Collections of 2.0 to 2.8 ml were made every 2 or 3 days starting on the day of estrus and continued until the next estrus. The blood was chilled and plasma was harvested by centrifugation at 4°. The plasma was stored at −20°.

Plasma progesterone assay. A competitive protein binding (CPB) procedure developed by Murphy (5) and modified by Neill *et al.* (6) for the assay of progesterone in peripheral plasma was used to quantitate progesterone in this study. The procedure used in this laboratory is described in more detail elsewhere (7). Into each plasma sample, progesterone-1,2-^{3}H (600 cpm 0.003 ng) in 100 μl of ethanol was flushed rapidly. Petroleum ether (5 ml) was added; and the tubes were shaken for 4 min. After centrifugation, the ether extract was pipetted into centrifuge tubes, the ether was evaporated, and 100 μl of chloroform were added. The extract was chromatographed by TLC in benzene: ethyl acetate (4:1, v/v). The portion of the plate containing progesterone was eluted with methanol (5 ml) into a tube and evaporated.

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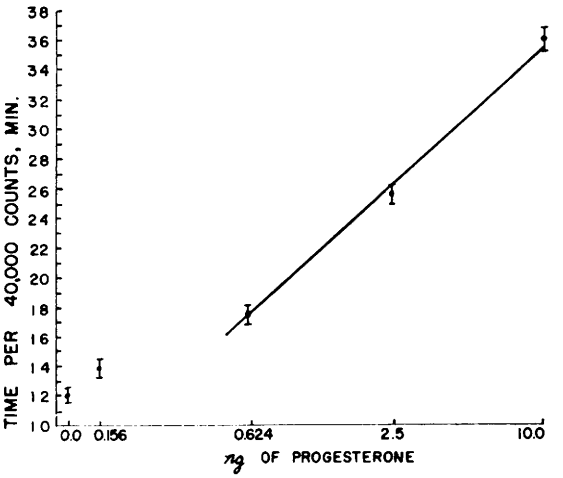

FIG. 1. A standard curve between 0.62 and 10.0 ng of progesterone on a log scale. The points are means of duplicates.

One milliliter of methanol was added and 0.5 ml was removed for counting progesterone-^{3}H. The remainder was evaporated and the contents in the tube were assayed as described by Neill *et al.* (6). The value obtained upon assay was adjusted according to the recovery of progesterone-^{3}H.

Plasma from a male dog was used as the source of binding protein. Corticosterone-1, 2-^{3}H (sp act 50.0 Ci/mmole), and progesterone-1,2-^{3}H (sp act 55.0 Ci/mmole) were obtained from New England Nuclear Corporation. All solvents were reagent grade.

The reliability of the procedure for assay of progesterone in guinea pig plasma was studied. Plasma from ovariectomized guinea pigs gave values which ranged from 0.20 to 0.46 ng of progesterone/ml. This constituted the blank value and in each assay a blank value was obtained and subtracted from the values obtained for the plasma samples. In a preliminary experiment, 1, 2, and 4 ng of progesterone were placed in tubes in duplicate. To each of the six tubes, ovariectomized plasma was added and assayed for progesterone. In comparison with the three quantities added, 1.26, 2.06, and 3.80 ng were recovered. In the assays of the plasma samples, an internal control was included. It consisted of distilled water to which 2.5 ng of progesterone were added. Values from the control tubes from all the assays gave a mean $\pm$ SD of 2.69 $\pm$ 0.57 ng/ml. Periodically an ovariectomized sample was also run. All plasma samples were run in duplicate using 0.25 ml of plasma. Progesterone content of a sample was read directly from a curve in which 4 doses on a log scale (0.156, 0.624, 2.5, and 10 ng) were plotted against minutes to reach a present count (40,000). The regression was linear over a range extending from 0.5 to 10.0 ng (see Fig. 1).

Ten guinea pigs which received sham operations and 10 animals which received IUFB were bled every 2 to 3 days beginning on the day of estrus. Some were bled through two estrous cycles to give a total of 13 cycles for the control group and 15 cycles for the treated group. When blood was collected from a guinea pig during two cycles, the cycle length and progesterone concentration patterns were similar between cycles.

The animals were sacrificed and the uteri were examined for presence of both IUFB and appearance of the tissue and lumen. One guinea pig had lost the IUFB in one horn, so its blood samples were discarded. The significance of differences in mean progesterone concentrations between days of the treatments and in lengths of the estrous cycle was determined by Student's *t* test.

Results. The progesterone concentrations in peripheral plasma from sham-operated animals and animals containing IUFB in both uterine horns during the estrous cycle are shown in Table I. The concentration in the

TABLE I. Plasma Progesterone Concentration in Guinea Pigs with IUFB and Sham-Operated Guinea Pigs.

Stage of cycle (days)	Sham animals		IUFB animals	
	No.	(ng of prog./ml of plasma)	No.	(ng of prog./ml of plasma)
0–1	10	0.20 ± 0.00[a]	12	0.20 ± 0.00
2–3	10	2.26 ± 0.30	9	1.54 ± 0.45
4–5	11	2.45 ± 0.23	11	2.60 ± 0.41
6–7	9	2.60 ± 0.28	10	2.23 ± 0.37
8–9	10	2.99 ± 0.23	11	1.91 ± 0.31
10–11	10	3.77 ± 0.47	11	1.19 ± 0.47
12–13	12	2.47 ± 0.22	7	0.40 ± 0.15
14–16	8	0.56 ± 0.32		

[a] Mean ± SE.

sham-operated group rose sharply by day 2 reaching 60% of the peak value obtained during the cycle. The concentration then rose at a slower rate to a peak value on day 10. Concentration fell almost 1 ng/ml between days 10 and 12 and 2 ng/ml between days 12 and 14. It reached a nondetectable level on day 16. The mean length of the estrous cycle in this group was 16.5 days (range, 15–18 days).

The progesterone concentration in the group having IUFB's rose at a more linear rate between days 0 and 4 than in the sham-operated animals. The peak concentration occurred on day 4. The concentration then gradually decreased over the next 8 days to a nondetectable concentration. By day 8 of the cycle, the concentration was significantly greater ($p<0.01$) in the sham-operated group than in the IUFB group. The mean length of the estrous cycle in this group was 12.7 days (range, 10–14 days). The difference between the mean lengths of the estrous cycle of the two groups was significant ($p<0.01$).

The uteri from the IUFB group appeared to have thicker endometrium and more fluid in the uterine lumen than the uteri from the sham-operated group. In some animals, the fluid was mostly confined to the IUFB, while in others, half the uterine horn was filled.

Discussion. Significant changes in peripheral plasma progesterone concentrations were obtained during the estrous cycle. It appears that the adrenal gland was not a significant source of the hormone, since concentrations were undetectable at the time near estrus, nor was the value from ovariectomized plasma greater than 0.5 ng/ml. It is pertinent that, in another study, progesterone concentration dropped sharply after ovariectomy to undetectable levels (8). The concentrations obtained during the cycle agree well with those reported earlier by Feder *et al.* (8) in which GLC was the method of progesterone assay, with one exception. In this study, concentrations were greater during the first 4 days of the cycle, suggesting that the CL were undergoing rapid functional development. A similar conclusion has been reached in other reports. In a study (1) of the mean volume of CL during the estrous cycle, the most rapid change occurred during the first 5 to 7 days. The pattern of plasma progesterone concentrations obtained in this study parallel the plot of mean volume of CL during the cycle reported by those workers. In addition, it was shown by Heap *et al.* (9) that progesterone concentration in luteal tissue reached a maximum concentration by days 5 or 6, and this concentration was maintained through day 9 after which it declined.

Plasma progesterone concentrations were significantly affected by an IUFB in both horns of the uterus. The results indicate that functional development of the CL during the first 4 days was as rapid as in the sham-operated animals. However, instead of increasing gradually between days 4 and 10 as in the sham-operated animals, the concentrations began to decline after day 4, indicating that the IUFB had arrested luteal function. Estrus occurred 6 to 9 days after the decline

began in the IUFB group, while 5 to 7 days elapsed after the decline began in the other group. The effect of an IUFB on growth of the CL, as measured by changes in the volume, was not detected until day 9, after which there was an abrupt decrease in volume (1). The timing of the IUFB effect on luteal function appears to be 5 to 6 days earlier. It should be noted, however, that the physical characteristics of the IUFB could cause a quantitative difference in the timing of the response. The lack of a continued rise in the progesterone concentration may be due to an inhibition of secretion while CL continue to grow. The fact that the length of the estrous cycle in animals with IUFB was similar to those reported in other studies (1, 2) suggests that a similar effect on the CL was being exerted by the IUFB.

Summary. The progesterone concentration in peripheral plasma of sham-operated guinea pigs increased significantly during the estrous cycle from a nondetectable concentration at estrus to a maximum concentration on day 10, followed by a rapid decline. In the presence of an IUFB in both uterine horns, the progesterone concentration did not increase after day 4 of the cycle. Over the next 8 days, a slow decline in concentration occurred. The length of the estrous cycle was significantly shorter in animals having IUFB than in sham-operated animals.

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