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Rat Placental Luteotropin: Initial Secretion and Luteolytic Quality[1] (42641)

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Abstract. The secretion of placental lactogen begins early in pregnancy. Previous studies indicate that rat placental lactogen (rPL) is secreted from Day 8 of pregnancy and that it is luteolytic as well as luteotrophic. This study establishes the onset of both the luteotrophic and the luteolytic effects of placental lactogen in pregnant rats subject to timed hypophysectomy. Pregnancy was preserved in all groups with the administration of dydrogesterone (9β, 10α-pregna4,6-diene-3, 20 dione), a progesterone analog, and diethylstilbestrol, an estrogen analog. Plasma progesterone and 20α-hydroxypregn-4-ene-3-one (20-OHP) were measured in serial serum samples by RIA. The data indicate that rPL is secreted as early in pregnancy as the seventh day. Rats hypophysectomized on Day 6 of pregnancy or later had ovaries that contained corpora lutea that secreted increasing quantities of progesterone during pregnancy. On Day 16 serum progesterone values were lowest in animals operated on Days 4 and 5 compared to animals operated on Days 6 or 8. The 20-OHP serum values from animals operated on Days 4 and 5 declined steadily from Day 8 to Day 16. These findings indicate progestational incompetency, which was confirmed morphologically. Thus, rPL secretion begins by Day 7 and it is both luteotrophic and luteolytic.

We have observed that pregnant–hypophysectomized rats had smaller ovaries if the corpora lutea no longer secreted progesterone (1, 2). Histological examination revealed the ovaries contained fewer and smaller corpora lutea which had few larger blossom cells and many cells with pyknotic nuclei in comparison to controls. This observation leads to the suggestion of luteolytic effects of placental luteotropin (rPL) as first described by Alloiteau (3) and of prolactin as described by Malven and Sawyer (4). Our radioimmunoassay of progestins in serum from pregnant–hypophysectomized rats demonstrated a lack of progesterone and an initial increase in 20α-hydroxypregn-4-ene-3-one (20-OHP) followed by a decline through successive daily samples. This latter pattern of 20-OHP was interpreted to be the result of structural luteolysis due to the presence of rPL. There is ample evidence, both biological (5) and biochemical (6), that rPL is present on Day 8 or 9 of pregnancy. However, the day of initial rPL secretion has not been determined.

The current study was begun to determine the earliest point in time that rPL begins to support the corpus luteum of pregnancy and to document the luteolytic effect of rPL. Our work was initiated with the assumption that rPL and prolactin functioned similarly and with the knowledge that following the absence of prolactin for 24 hr the administration of luteotropin will not reinstitute progesterone secretion (7).

Materials and Methods. Two- or three-month-old Sprague–Dawley rats were kept in a light- and temperature-controlled room. Females were caged with experienced males for breeding. Vaginal washes were examined each morning and the day sperm were observed was defined as Day 1 of pregnancy. Pseudopregnancy was induced by tapping the uterine cervix 25 times with a glass rod on the evenings of proestrus and estrus. Day 1 of pseudopregnancy (the day of estrus) was defined retrospectively from the day of metestrus determined from microscopic examination of vaginal washings. Parapharyngeal hypophysectomies of pregnant rats were performed on Days, 4, 5, 6, and 8. Pseudopregnant rats were hypophysectomized on Days

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TABLE I. TISSUE WEIGHTS AND FETAL SURVIVAL IN PREGNANT RATS

APX[a] (Day)	n	Ovary (mg)[b]	Adrenal (mg)	Mean fetal (mg)	Mean placenta (mg)	Percentage survival[c]
—	3	109 ± 8	88 ± 8	265 ± 42	347 ± 58	97 ± 3
4	8	31 ± 2	38 ± 2	253 ± 4	368 ± 18	84 ± 9
5	5	29 ± 3	27 ± 2	230 ± 12	375 ± 39	91 ± 9
6	6	60 ± 7	34 ± 1	261 ± 10	384 ± 12	96 ± 2
8	10	65 ± 6	34 ± 2	245 ± 12	332 ± 19	95 ± 2

Note. Rats were hypophysectomized on Days 4, 5, 6, or 8 and given 3 mg dydrogesterone plus 200 ng DES daily through Day 12. All rats were sacrificed on Day 16.

[a] APX, hypophysectomy.

[b] Tissue weight, mg ± SEM.

[c] Percentage fetal survival, live/total × 100.

4, 6, and 8. Completeness of the surgery was judged at sacrifice on Day 16 by examination of the sella turcica with a dissecting loupe and determination of adrenal gland weight loss. Pregnancy and pseudopregnancy were preserved by administering dydrogesterone (9β, 10α-pregna-4,6-diene-3, 20-dione), 3 mg/day, and diethylstilbestrol (DES) [(E)-4,4′-(1,2 diethyl-1,2 ethenediyl) bisphenol], 200 ng/day, from the day of hypophysectomy through Day 12. Serial blood samples were obtained by orbital sinus puncture. Terminal samples were obtained by exsanguination. All blood samples were heparinized and centrifuged. The plasma was decanted and frozen until analysis for progesterone and 20-OHP by RIA (2). Fetal viability was established at autopsy by observing heartbeats in each fetus. Fetal and maternal tissues were dissected from extraneous tissue and weighed. Pseudopregnant rats were sacrificed on Day 16 and ovarian, adrenal, and uterine weights were determined.

Data were analyzed by analyses of variance. Steroid values were grouped according to the day of sampling and analyzed.

Results. Hypophysectomy caused ovarian and adrenal weights to be reduced ($P < 0.01$). The ovarian weights of pregnant animals fell into two separate and different groups ($P < 0.01$), one operated on Days 4 and 5 and the other operated on Days 6 and 8. The weights were similar within groups. Those operated on Days 4 and 5 were smaller than those operated on Days 6 and 8 (Table 1). Fetal weights, placental weights, and percentages of fetal survival were not different due to treatment.

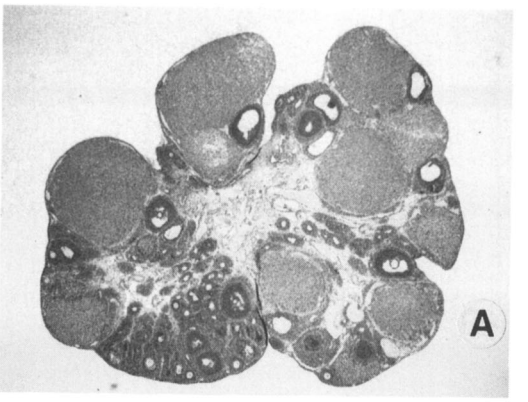
Hypophysectomy of pseudopregnant rats reduced adrenal ($P < 0.01$) and ovarian weights ($P < 0.01$) (Table II). Observation of histological sections of ovarian tissue from pregnant and pseudopregnant rats showed ovaries from pregnant rats operated on Days 4 and 5 lacked substantial amounts of luteal tissue (Fig. 1). In contrast, the sections from ovaries of pregnant rats operated on Days 6 and 8 had more and larger corpora lutea which appeared more normal. The cells lacked pyknotic nuclei and contained more cytoplasm. The corpora lutea from ovaries of pregnant rats operated on Day 8 were more robust than those from animals operated on Day 6. Comparison of control ovaries obtained from pseudopregnant rats on Days 4, 6, and 8 with those from rats hypophysectomized on Days 4, 6, and 8 of pseudopreg-

TABLE II. OVARIAN AND ADRENAL WEIGHTS OF PSEUDOPREGNANT RATS KILLED OR HYPOPHYSECTOMIZED (APX) ON DAYS 4, 6, OR 8

Day	Treatment	n	Tissue weight (mg)[a] Ovary	Adrenal
4	Sacrificed	3	78 ± 8	73 ± 7
4	APX	3	37 ± 1	28 ± 2
6	Sacrificed	3	81 ± 8	67 ± 5
6	APX	3	49 ± 6	34 ± 1
8	Sacrificed	3	80 ± 7	67 ± 2
8	APX	3	46 ± 6	36 ± 3

Note. APX rats were given 3 mg dydrogesterone plus 200 ng DES daily through Day 12 and sacrificed on Day 16.

[a] Mean ± SEM.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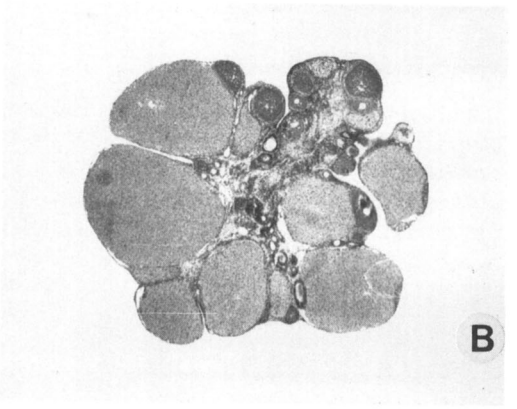
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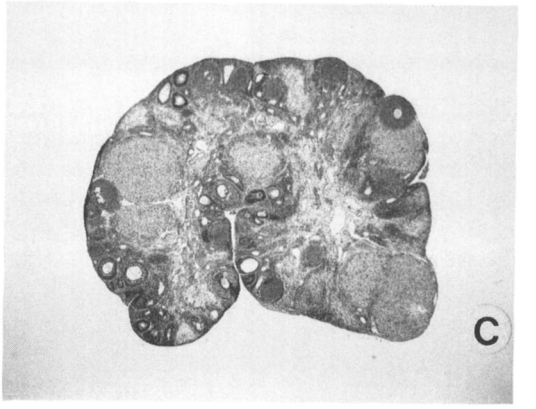
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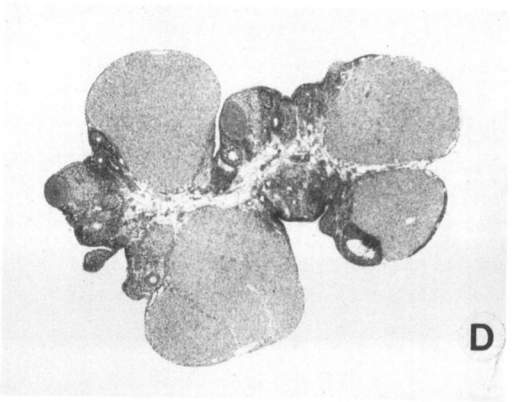
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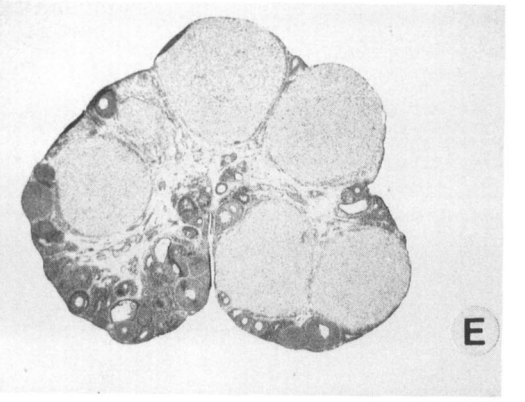
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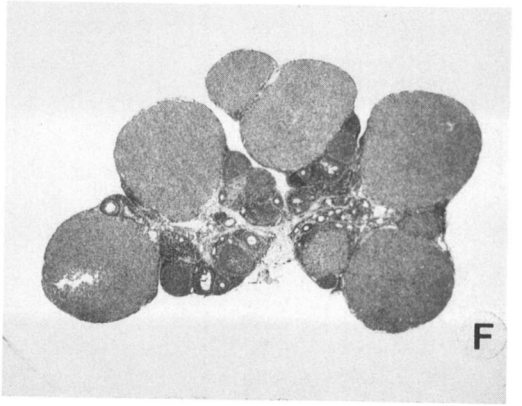
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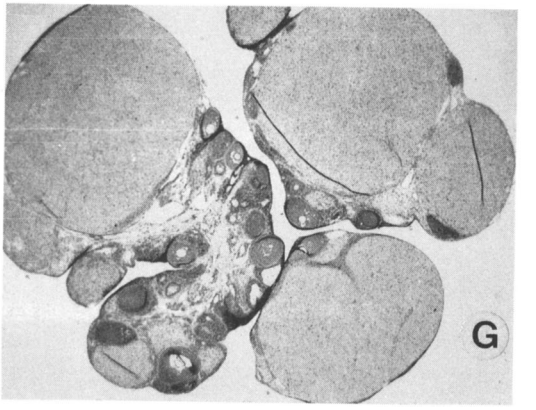
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TABLE III. SERIAL PLASMA PROGESTIN LEVELS (ng/ml) IN PSEUDOPREGNANT RATS FOLLOWING HYPOPHYSECTOMY

	APX (Day)	n	Day of pseudopregnancy: 6	8	10	12	14	16
Progesterone	4	3	3.7 ± 0.7	4.0 ± 0.4	4.2 ± 0.6	3.5 ± 0.4	2.4 ± 0.2	3.1 ± 0.7
(ng/ml plasma	6	3		5.4 ± 1.0	6.2 ± 1.4	5.2 ± 1.5	3.5 ± 0.8	2.9 ± 0.7
± SEM)	8	3			4.8 ± 0.3	4.2 ± 0.8	4.3 ± 0.4	3.3 ± 1.0
20-OHP	4	3	56.0 ± 7.7	76.0 ± 13.8	63.7 ± 14.6	65.6 ± 12.8	60.0 ± 6.1	54.2 ± 2.9
(ng/ml plasma	6	3		94.7 ± 6.9	101.5 ± 9.6	89.9 ± 16.6	70.7 ± 17.9	40.5 ± 5.5
± SEM)	8	3			89.5 ± 9.6	86.1 ± 16.1	88.7 ± 13.6	63.0 ± 9.1

nancy and subsequently treated with dydrogesterone and DES until Day 12 and sacrificed on Day 16 revealed a loss of tissue size rather than ovarian elements. This was in sharp contrast with the ovaries of pregnant animals operated on Days 4 and 5 which had very little luteal tissue, indicating the luteolytic action of rPL. Hypophysectomy reduced the number and size of antral follicles in all ovaries.

Plasma progesterone values from pseudopregnant rats were low, less than 6.2 ng/ml, and did not differ within time periods (Table III). The 20-OHP values were not different within time periods except for one (Day 16, 40 ng/ml, $P < 0.01$). Plasma progesterone values from pregnant rats hypophysectomized on Days 4 and 5 were only different on Day 14 ($P < 0.05$). These plots show low progesterone levels until Day 10, an increase to Days 12 and 14, followed by a sharp fall to Day 16 (Fig. 2A). The plasma progesterone values from rats operated on Day 6 followed a pattern similar to those operated on Days 4 and 5. Day 8 and 10 values were low and increased on Days 12 and 14. However, on Day 16 the values increased further and were greater than the values from animals operated on Days 4 and 5 ($P < 0.01$). The progesterone values from animals operated on Day 8 were greater on Day 10 than all other groups ($P < 0.01$) and increased on each day thereafter.

The plasma 20-OHP values of pregnant animals hypophysectomized on Days 4 and 5 were different on Day 14 ($P < 0.05$). However, they described the same trend, a continuous decline from Day 8 to Day 16 (Fig. 2B). The 20-OHP values from pregnant animals operated on Days 6 and 8 were different on Day 10. The values held constant on Days 12, 14, and 16.

The plasma progesterone values from pseudopregnant rats hypophysectomized on Days 4, 6, and 8 were low in comparison with plasma values from pregnant rats and not different from each other. The plasma 20-OHP values from pseudopregnant animals operated on Days 4 and 8 were not different within groups. The 20-OHP values from pseudopregnant animals operated on Day 6 were different ($P < 0.05$) due to the reduced value on Day 16 compared to all other days ($P < 0.01$).

Discussion. It is evident from the ovarian weight differences, the histological appearance of the ovaries, and the patterns of change in plasma progestin concentrations that morphological luteolysis occurred in pregnant rats hypophysectomized on Days 4 or 5 presumably due to the presence of a product of the placenta. Luteolysis only occurred if the rats were hypophysectomized before rPL secretion began and with sufficient time lapse to allow the corpora lutea to become nonresponsive to the luteotrophic ef-

FIG. 1. Photomicrographs of histological sections from ovaries of Control (A) hypophysectomized–pseudopregnant, or -pregnant rats (B–G). Operated animals were treated with dydrogesterone, 3.0 mg daily, plus DES, 200 ng daily, from the day of surgery until Day 12. They were sacrificed on Day 16. (A) From an animal sacrificed on Day 4 of pseudopregnancy; (B, D, F) from pseudopregnant rats; (C, E, G) from pregnant rats. The rats were hypophysectomized on Days 4 (B, C), 6 (D, E), and 8 (F, G). (magnification 11.8×).

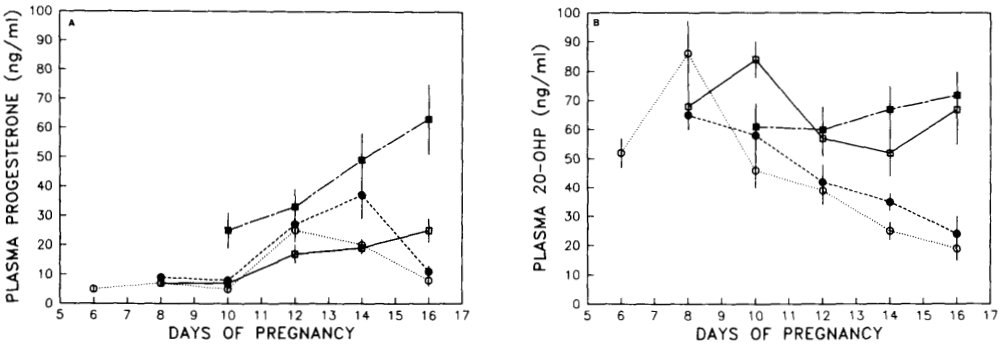

FIG. 2. Rat plasma progesterone (A) and 20-OHP (B) values (ng/ml ± SEM) derived from groups of rats that were bled on the days indicated. Therefore, each point is composed of values obtained from the same rats within a group. Animals were hypophysectomized on Day 4, $n = 8$ (○); Day 5, $n = 5$ (●); Day 6, $n = 7$ (□); or Day 8, $n = 10$ (■). Their pregnancies were supported with dydrogesterone, 3.0 mg daily, plus DES, 200 ng daily, from the day of surgery through Day 12. They were sacrificed on Day 16.

fect of rPL. When these conditions were met progesterone secretion was reduced and 20-OHP secretion increased initially and then declined sharply. This resulting continuous decline of 20-OHP secretion is considered a measure of luteolysis, since neither pregnant animals with continuing progesterone and 20-OHP secretion nor pseudopregnant rats with continued 20-OHP secretion exhibited a similar decline, qualitative or quantitative. The effect of luteal regression was also monitored by the final low ovarian weights of animals hypophysectomized on Days 4 and 5 as compared to animals operated on Days 6 and 8. The ovarian weight loss was due to the reduced quality and quantity of luteal tissue found upon histological examination, and which ultimately caused the pattern changes in plasma progestin concentrations.

Conversely, the luteotrophic effect of rPL on corpus luteum progesterone secretion was evident in rats hypophysectomized on Days 6 and 8. At autopsy their ovaries weighed more and retained more corpora lutea composed of competent appearing cells which secreted more progesterone than their counterparts operated on Days 4 and 5.

The ability of prolactin to support or lyse corpora lutea depends upon a 24-hr time lag between the previous luteotrophic support and the onset of the replacement therapy (7). In this study, removal of prolactin by hypophysectomy allowed regression of the whole ovary in pseudopregnant rats with no luteal lysis. However, corpora lutea were lysed in pregnant rats that experienced a void of luteotrophic support before the placenta began to secrete rPL. In contrast, those corpora that did not experience the void in support retained their progesterone secretory capacity and persisted. With the assumption that pituitary prolactin and rPL act in a similar manner on the same mechanism one can speculate that rPL is available to the corpus luteum of pregnancy as early as Day 7, since the corpora lutea of animals operated on Days 4 or 5 lysed and those of animals operated on Day 6 survived and functioned.

The original design of these experiments incorporated a lapse of steroid analog therapy between Day 12 and Day 16 to allow fetal abortion/resorption if luteal function had ceased. However, fetal abortion/resorption did not occur and although progesterone values were quite reduced a high percentage of fetuses were viable at autopsy. In this experimental situation failure of the corpus luteum may not be as precipitous as ovariectomy. It is possible that the fetuses of animals operated on Day 4 or 5 would not have endured to term because the steroid analog therapy may have provided only enough support to maintain the fetuses to Day 16. The lower fetal survival and increased standard errors observed on Day 16 may be the foreshadowing of this fetal loss.

Speculation allows that the placenta produced the progesterone measured in the

plasma of animals operated on Day 4 or 5. There is no doubt as to fetoplacental progesterone secretion (8). However, the fetoplacental unit is not considered the only source on Day 14, due mainly to the disparity of the values on that day. Other sources of progesterone could be the result of interaction between the fetus and the maternal adrenal, or from the fetal gonad or adrenal.

In review of this subject one cannot overlook the newly suggested decidual luteotrophic factor (9). It is possible that this material could act with rPL to aid the luteotrophic effects or that it is a luteotrophic agent. However, this uterine factor requires the presence of an LH-like material to express its luteotrophic effect. Since hypophysectomy excludes a LH-like factor and the luteotrophic effect of the placenta was first shown by injecting masserates of Day 12 placenta (10), it seems likely that the effects observed in this study are the result of rPL.

Thus, rat placental lactogen is luteolytic and luteotrophic. Its secretion begins shortly after implantation and from these data as early as Day 7.

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