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# Inhibitory Effect of Charcoal-Treated Aqueous Porcine Corpus Luteum Extract upon Ovulation in the Rabbit (41094)[1]

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*Abstract.* Administration of charcoal-treated aqueous extract of porcine corpus luteum on Days 0, 1, and 2 after mating inhibited coitus-induced ovulation in the rabbit. Inhibition of ovulation was evidenced by a significant ($P < 0.01$) inhibition of the coitus-induced rise in serum progesterone and lack of formation of corpora lutea. Administration of charcoal-treated heart or lung extract had no significant effect upon the formation of corpora lutea or on the coitus-induced rise in serum progesterone. Administration of the same extract 3 and 4 days after coitus-induced ovulation had no significant effect upon serum progesterone and further formation of corpora lutea. These data are consistent with the existence of an LH binding inhibitor in porcine corpus luteum extract which acts to inhibit the ovulatory response to the coitus-induced LH surge.

We have shown that addition of charcoal-treated aqueous extract of porcine corpus luteum inhibits binding of labeled human chorionic gonadotropin (hCG) to porcine granulosa cells (1). Yang, Saaman, and Ward succeeded in isolating a luteinizing hormone (LH) binding inhibitor from luteinized rat ovaries (2). No *in vivo* evidence of inhibition of LH or hCG binding was observed, however. Therefore, the present studies were undertaken to see if charcoal-treated aqueous extract of corpus luteum could inhibit coitus-induced ovulation and corpus luteum function in the rabbit *in vivo*.

**Materials and Methods.** Thirty-seven New Zealand white sexually mature female rabbits were used for mating. Each rabbit was housed individually on a 12-h light 12-h dark cycle and fed Purina Rabbit Chow and given water *ad libitum*. Rabbits were checked daily for signs of estrus immediately upon arrival from the supplier. Rabbits having a deep purple vulva were judged to be in estrus. On the first day they had a purple vulva they were given an intraperitoneal injection of 5 ml of either charcoal-treated porcine lung, heart, or corpus luteum extract at 0 hr. Thirty minutes postinjection they were allowed to mate with a proven fertile buck (Day 0). Mating was observed during a 1-hr period and each female was observed until she mated successfully at least once. She was left with the male an additional 2–5 hr and separated. Rabbits that failed to mate during a 3- to 6-hr period were excluded from the study. On Days 1 and 2 after mating additional injections of lung or corpus luteum extract were given. In some instances daily 2- to 3-ml blood samples were taken from an ear vein from Day 0 to 7 or 10 of gestation. Additional rabbits were given either charcoal-treated heart or corpus luteum extract on Day 3 and 4 after mating.

On Day 7 or 10 of gestation rabbits were sacrificed and the reproductive tracts and ovaries were examined. The uterus was dissected out, slit longitudinally, and the number of viable and reabsorbing embryos was counted. A biopsy of each embryo was fixed in 10% formalin, processed histologically, and a representative section was stained with hematoxylin (H) and eosin (E). The ovaries were dissected free of connective tissue and fat and the number of corpora lutea and hemorrhagic follicles was counted. Subsequently the ovaries were fixed in 10% formalin and processed histologically. One ovary of each pair was serially sectioned and stained with H and E so

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that the corpora lutea and follicles could be counted.

Serum progesterone content was measured by radioimmunoassay in duplicate aliquots of serum samples after extraction with petroleum ether (3, 4). A specific 11-OH progesterone–bovine serum albumin conjugate antiserum generated in a sheep was employed in the assay and was generously donated by Dr. Gordon Niswender.

The extract of corpus luteum was prepared as detailed by us previously (5). Essentially mid- and late-luteal-phase porcine corpora lutea were homogenized in 6 vol of water followed by centrifugation at 30,000*g* and treatment with 1% charcoal by volume. The extract was passed through a UM05 Amicon membrane until the extract was reduced two to three times its original volume. Two pools of corpus luteum extract were employed and an aliquot of each was assayed for ability to inhibit binding of $^{125}$I-hCG to porcine granulosa cells as detailed by us (1). Both pools contained significant hCG binding inhibitory activity such that 50 $\mu$l of a one-fifth dilution of the extract inhibited the binding of 60–70% of 5 ng $^{125}$I-hCG. The progesterone content of both charcoal-treated corpus luteum extracts was determined and found to be 90–100 $\mu$g/ml. The charcoal-treated heart and lung extracts had no detectable hCG binding inhibitory activity when assayed undiluted. All extracts were used after at least 2 months of storage at −20° since it has been found by us that storage for 1.5 weeks or longer leads to appearance of the inhibitory activity on hCG binding. Data were analyzed using Student's *t* test.

**Results.** As shown in Table I administration of 5 ml of charcoal-treated aqueous extract of porcine corpus luteum (LHRBI) to 12 rabbits on Days 0, 1, and 2 postcoitum inhibited ovulation as evidenced by absence of corpora lutea 7 days after mating. In contrast, rabbits given lung extract on Days 0, 1, and 2 postcoitum ovulated as evidenced by observation of corpora lutea (6–12 per two ovaries) on Day 7 after mating (Table I). Serum progesterone levels were elevated compared to LHRBI-treated rabbits ($P < 0.01$). Nine of the lung extract-treated mated rabbits selected at random had three to six embryos. Another two series of control rabbits were given heart extract on Days 0, 1, and 2 and on Days 3 and 4 after mating and allowed to go to term. Blood samples were taken daily or on alternate days up until Day 10 after mating in order to establish a control secretion pattern of progesterone which is summarized in Tables II and III. It should be noted that administration of heart extract on Days 0, 1, and 2 (Table II) exerted a transitory significant decrease in serum progesterone ($P < 0.05$) after 1 and 2 days compared to rabbits given heart extract after 3 and 4 days (Table III). The differences in serum progesterone between these two groups of heart-treated rabbits were not statistically different after 2 days.

Four rabbits given heart extract on Days 0, 1, and 2 after mating had serum progesterone levels consistent with a normal luteal function (Table II). Both heart and corpus luteum extracts were also given at Days 3 and 4 after mating to test for any effects at this time on pregnancy or luteal function as reflected by changes in serum progesterone levels. Of the seven rabbits given heart extract on Days 3 and 4, six rabbits delivered four to six young at term and the seventh delivered no young and did not ovulate as evidenced by a lack of corpora lutea. Data from this rabbit are not averaged in with the rest on Table III. An additional five rabbits were treated with LHRBI on Days 3 and 4 after mating, and permitted to go to Day 10 after mating and autopsied. Data for these rabbits are shown in Table III. Four rabbits showed evidence of ovulation, and contained 4–17 corpora lutea. One rabbit failed to ovulate. The LHRBI did not significantly ($P > 0.05$) alter circulating progesterone levels compared to heart extract-treated rabbits, although there was a nonstatistically significant tendency to decrease progesterone levels (50–60%) during the third and fourth days of treatment.

**Discussion.** These data demonstrate an inhibitory action of a crude charcoal-treated aqueous extract of porcine corpus luteum upon ovulation in the rabbit. The

TABLE I. EFFECT OF CHARCOAL-TREATED CORPUS LUTEUM AND LUNG EXTRACT GIVEN ON DAYS 0, 1, AND 2 AFTER MATING UPON SERUM PROGESTERONE, CORPORA LUTEA, AND IMPLANTATION

| Days after mating | Serum progesterone (ng/ml) LHRBI-treated Days 0, 1, 2 Mean | SE | No. Obs. | Lung extract-treated Days 0, 1, 2 Mean | SE | No. Obs. | *P* value lung vs LHRBI treated |
|---|---|---|---|---|---|---|---|
| 0 | 0.46 | 0.20 | 5 | 2.16 | 0.51 | 4 | <0.01 |
| 1 | 0.61 | 0.32 | 5 | 2.29 | 0.36 | 3 | <0.01 |
| 2 | 0.23 | 0.14 | 3 | 2.6 | 0.20 | 3 | <0.001 |
| 3 | | | | | | | |
| 4 | 0.17 | 0.03 | 3 | | | | |
| 5 | | | | | | | |
| 6 | 0.11 | 0.03 | 3 | | | | |
| 7 | 0.48 | — | 2 | 5.0 | 0.89 | 5 | |

| | LHRBI-treated Days 0, 1, 2 | Lung extract-treated Days 0, 1, 2 |
|---|---|---|
| Number corpora lutea | | |
| Left | 0 (12)[a] | 3–8 (9) |
| Right | 0 (12) | 3–6 (9) |
| Total | 0 (12) | 6–12 (9) |
| Number implantations | | |
| Left | 0 (12) | 2–3 (9) |
| Right | 0 (12) | 0–3 (9) |
| Total | 0 (12) | 3–6 (9) |

*Note.* Five milliliters of charcoal-treated corpus luteum extract (LHRBI) or lung extract was administered intraperitoneally 30 min prior to mating and on Days 1 and 2 after mating to rabbits. After 7 days the rabbits were autopsied and the number of corpora lutea and blastocysts was counted. Rabbits were sampled for blood immediately after mating on Day 0 and daily thereafter as shown in the Table and progesterone was measured in the serum after extraction with petroleum ether.

[a] Number of rabbits indicated in parentheses.

data can best be explained by an inhibitory action of ovarian LH binding occurring during mating which was previously demonstrated *in vitro* by us (1) and by Yang and his colleagues (2). If the crude luteal extract was given later during gestation on Days 3 and 4 it did not inhibit implantation demonstrating that the extract did not exert a nonspecific necrotic effect upon the ovary or conceptus.

The chemical component of the luteal extract responsible for inhibiting ovulation is not known since chemical purification of the active LH/hCG binding inhibitor has not been achieved. Studies by Yang and his colleagues (2) indicate that the LH binding inhibitor present in extracts of rat corpus luteum is polypeptide in nature and principally of low molecular weight. However, recent observations by Ward (6) using porcine corpora lutea extract indicate that there probably is both a high- and a low-molecular-weight form of the LH binding inhibitor. The possibility exists that it is a fragment of LH or an altered LH receptor. The additional possibility that contaminating progesterone or other steroid which was not removed by the charcoal treatment was responsible for inhibition of ovulation was not rigorously ruled out in these studies. It is unlikely, however, that the contaminating progesterone itself was responsible for inhibition of ovulation. Previous studies carried out by Sawyer and Everett in which 2 mg of progesterone in oil sc was injected into rabbits 1–4 hr prior to mating and was

TABLE II. EFFECT OF HEART EXTRACT GIVEN ON DAYS 0, 1, AND 2 AFTER MATING UPON SERUM PROGESTERONE

| Days after mating | Serum progesterone (ng/ml) | | |
|---|---|---|---|
| | Mean | SE | *n* |
| −2 | 0.29 | 0.02 | 4 |
| −1 | 0.69 | 0.35 | 4 |
| 0 | 3.26 | 1.28 | 3 |
| 1 | 0.66 | 0.14 | 4 |
| 2 | 1.38 | 0.17 | 4 |
| 3 | 2.98 | 0.73 | 3 |
| 4 | 3.24 | 0.82 | 3 |
| 5 | 4.36 | 0.72 | 3 |
| 6 | 6.70 | 1.84 | 3 |
| 7 | 5.79 | 1.28 | 4 |
| 8 | 6.88 | 1.83 | 4 |

*Note.* Five milliliters of charcoal-treated heart extract was administered intraperitoneally 30 min prior to mating and on Days 1 and 2 after mating to four rabbits. Daily blood samples were obtained and assayed for progesterone.

found not to inhibit mating-induced ovulation may rule out an effect of progesterone itself upon ovulation. It is possible that much of the progesterone in the luteal extracts was bound to proteins present in the crude luteal extract rendering the amount of free progesterone available to act upon target tissue less than that found if the progesterone were given in oil. Since progesterone determination on the extract was carried out after the extract was extracted with petroleum ether the total progesterone rather than the "free" or "bound" progesterone was measured. If greater than 1% charcoal was used to remove the progesterone it was found that much of the LHRBI activity active *in vitro* was removed from the extract (5).

It is also possible that some components of the corpus luteum extract inhibited ovu-

TABLE III. EFFECT OF CHARCOAL-TREATED HEART EXTRACT AND CORPUS LUTEUM EXTRACT GIVEN ON DAYS 3 AND 4 AFTER MATING UPON SERUM PROGESTERONE, CORPORA LUTEA, AND IMPLANTATIONS IN THE RABBIT

| Days after mating | Serum progesterone (ng/ml) | | | | | |
|---|---|---|---|---|---|---|
| | LHRBI-treated Days 3, 4 | | | Heart extract treated Days 3, 4 | | |
| | Mean | SE | No. Obs. | Mean | SE | No. Obs. |
| 0 | | | | 2.42 | 0.93 | 5 |
| 1 | 4.5 | 1.7 | 4 | 3.14 | 0.89 | 5 |
| 2 | 1.6 | 0.37 | 4 | 3.49 | 0.54 | 5 |
| 3 | 2.4 | 0.48 | 4 | 4.67 | 0.73 | 6 |
| 4 | 2.6 | 0.30 | 4 | 4.89 | 1.10 | 6 |
| 5 | 4.5 | 1.3 | 4 | 5.2 | 1.37 | 4 |
| 6 | 4.7 | 0.67 | 4 | 6.4 | 1.6 | 6 |
| 7 | – | – | – | – | – | – |
| 8 | 5.9 | 0.88 | 4 | 10.3 | 3.2 | 4 |
| 9 | – | – | – | | | |
| 10 | 6.6 | 0.9 | 4 | | | |
| | Range | Avg | No. Obs. | Range | Avg | |
| Number corpora lutea | 4–17 | 10 | 4 | | | |
| Number embryos | 7–9 | 8 | 4 | | | |
| Number live young born at term | NA | NA | NA | 4–6 | 6 | |

*Note.* Five ml of charcoal-treated heart or corpus luteum extract was administered intraperitoneally 3 and 4 days after mating to six and four rabbits, respectively. Daily blood samples were obtained and assayed for progesterone. Some rabbits were allowed to go to term and others were autopsied after 10 days.

lation by effects on hypothalamic–pituitary function rather than by a direct effect upon the ovary. Further studies with more purified corpus luteum LH binding inhibitor will be required in order to determine the nature of the active ovulation-inhibiting component of the luteal extract.

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