EFFECTS OF EXOGENOUS PROGESTERONE ON CORPORA LUTEA INDUCED IN ANESTROUS EWES

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A negative feedback action on pituitary function is a possible mechanism whereby exogenous progesterone affects the corpus luteum (CL) of the ewe. This concept is supported by the reports of Zimbelman et al. (1959) and Woody et al. (1967). Other evidence suggests that progesterone may limit the life span of induced CL by an action exerted prior to the time of induced ovulation. The induction of new CL during diestrus did not extend estrous cycle length (Inskeep et al., 1963). Inskeep et al. (1964) suggested that the presence of progesterone at the time new CL were induced acted to reduce their life span. Since the life span of the induced CL was inversely related to the length of time the natural CL had been present (Inskeep et al., 1963), it is possible that progesterone exerted an action prior to the time the new CL were induced and their life span depended upon the length of time that progesterone had been present.

The present experiment was designed to compare the effects of progesterone on induced CL when the progesterone was injected either prior to ovulation or beginning on the day of ovulation, or injected at both times. The experiments were made with anestrous ewes to minimize the influence of endogenous progesterone.

Materials and Methods

In the late spring of 1965, treatment designed to cause ovulation on day 7 of the experimental period was given to 43 anestrous ewes. They had been allotted randomly to four experimental groups (table 1) and treated as follows: (1) no progesterone (controls), 10 ewes; (2) 25 mg. progesterone per day for 6 days prior to ovulation (days 1 to 6), 11 ewes; (3) 25 mg. progesterone per day for 6 days beginning on the expected day of ovulation (days 7 to 12), 11 ewes and (4) 25 mg. progesterone per day for 12 days beginning 6 days prior to ovulation (days 1 to 12), 11 ewes. The experiment was repeated in the late spring of 1966 when 40 ewes were allotted randomly to the four groups. Nonlactating ewes of several breeds and breed-crosses (of fine- and medium-wool types) were used in each of the 2 years.

The ewes were checked throughout their experimental period for estrus, using vasectomized rams. Rams either were painted and placed continuously with the ewes or turned in with the ewes once daily.

The progesterone (25 mg. per ml. in corn oil) was given subcutaneously. Ovulation was induced with a single preparation of beef pituitary extract. One gram equivalent, in 1 ml. saline, was injected intramuscularly on day 4 followed in 24 hr. (36 hr. in the first 12 ewes) by 5 gm. equivalents in 5 ml. saline given intravenously. Ovulation was expected to occur approximately on day 7. Mid-ventral laparotomies were performed on day 8. All ewes which had no CL, or in which CL were present that were covered by the serous layer ("old" CL, probably present prior to the ovulating treatment) were discarded. The newly forming CL in the remaining ewes were marked with India ink. The ewes were killed on day 13, 6 days after expected ovulation, and the CL were recovered and weighed.

The data for CL weights were analyzed by analysis of variance using the method of weighted means (Steel and Torrie, 1960). The average CL weights for the four experimental groups were compared using Duncan's (1955) multiple range test as modified by Kramer (1956) for unequal numbers. Where more than one CL was present in a ewe, the average CL weight was used.

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2 The advice of Dr. L. E. Casida, especially in planning the experiments, is gratefully acknowledged.

3 Extract prepared by J. Riesen by a modification of the method of Koenig and King (1950). Frozen pituitaries were extracted and 10.34 mg. extract = 1 gm. pituitary tissue.
TABLE 1. EXPERIMENTAL DESIGN AND PROCEDURES FOR STUDYING EFFECTS OF PROGESTERONE ON INDUCED CL IN ANESTROUS EWES

<table>
<thead>
<tr>
<th>Day of experiment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>IM</td>
<td>IV</td>
<td>.</td>
<td>O</td>
<td>L</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>K</td>
</tr>
<tr>
<td>Day 1 to 6</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Day 1 to 12</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

Additional treatment of individual groups:
- IM = intramuscular injection of pituitary extract
- IV = intravenous injection of pituitary extract
- O = expected time of ovulation
- L = laparotomy
- K = kill
- P = progesterone

Results and Discussion

Of the 83 ewes, seven (8.4%) were discarded because "old" CL were present at the time of laparotomy, although five (71.4%) of these ewes also had newly forming CL. Of the 76 ewes remaining, 58 (76.3%) ovulated and had an average of 1.64 (range 1 to 3) newly forming CL at the time of laparotomy (table 2). After examination of the data from the first year, it appeared that there might be differences in ovulation rate among the four treatment groups. However, this observation was not confirmed by the results in the second year (P > .25). No ewes were detected in heat during the experimental period.

Analysis of variance of the weights of the CL showed there was an effect of treatment (P < .005), but not of year, nor interaction of treatment and year. Data for the 2 years were combined and the group means were compared (table 2). The average CL weight was lower (P < .01) in ewes treated with progesterone on days 1 to 6 or on days 1 to 12 than in the control ewes or in ewes treated with progesterone on days 7 to 12. There was no significant difference between the average CL weight of ewes injected on days 1 to 6 and of those injected on days 1 to 12, nor was there such a difference between the average CL weight of the control ewes and of those that were injected with progesterone on days 7 to 12. The number and percent of ewes having marked CL that were far-regressed, as judged by their color and size, were: control, 5 (35.7%); progesterone days 1 to 6, 9 (64.3%); progesterone days 7 to 12, 1 (6.7%); progesterone days 1 to 12, 13 (86.7%). One ewe in the last group had a marked, non-regressed CL as well as a marked, regressed one.

These results do not support the hypothesis that progesterone exerts its action on CL of ewes by a negative feedback action on pituitary function. It has been suggested recently, based on the effects of hypophysectomy, that CL of ewes are dependent on pituitary support for at least 5 days after ovulation (Kaltenbach et al., 1966). If this condition applies to induced CL of anestrous ewes, then, in the ewes injected with progesterone beginning on the day of ovulation, the CL should have been reduced in weight. However, in ewes so injected, the CL were not smaller than those

TABLE 2. EFFECT OF PROGESTERONE ON THE WEIGHT OF CL INDUCED IN ANESTROUS EWES

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Year</th>
<th>No. ewes</th>
<th>Av. no. CL</th>
<th>Av. CL wt. (mg.)</th>
<th>Combined av. CL wt. (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1965</td>
<td>7</td>
<td>1.57</td>
<td>217.1</td>
<td>260.6*</td>
</tr>
<tr>
<td></td>
<td>1966</td>
<td>7</td>
<td>1.28</td>
<td>304.0</td>
<td></td>
</tr>
<tr>
<td>Day 1 to 6</td>
<td>1965</td>
<td>7</td>
<td>1.00</td>
<td>115.6</td>
<td>117.5*</td>
</tr>
<tr>
<td></td>
<td>1966</td>
<td>7</td>
<td>1.71</td>
<td>119.4</td>
<td></td>
</tr>
<tr>
<td>Day 7 to 12</td>
<td>1965</td>
<td>7</td>
<td>2.28</td>
<td>324.5</td>
<td>329.5*</td>
</tr>
<tr>
<td></td>
<td>1966</td>
<td>8</td>
<td>1.75</td>
<td>333.9</td>
<td></td>
</tr>
<tr>
<td>Day 1 to 12</td>
<td>1965</td>
<td>7</td>
<td>2.00</td>
<td>60.5</td>
<td>90.4*</td>
</tr>
<tr>
<td></td>
<td>1966</td>
<td>8</td>
<td>1.50</td>
<td>116.6</td>
<td></td>
</tr>
</tbody>
</table>

* Error mean square=18.906.

** Mean weights not having the same letter in the superscript differ significantly (P<.01).
of control ewes. In contrast, the injection of progesterone prior to the time of ovulation reduced CL weight, whether or not the injections were continued after ovulation. Thus, an action of progesterone was exerted prior to the time of ovulation which resulted in reduced CL weight 6 days after ovulation.

**Summary**

The effects of exogenous progesterone on corpora lutea induced in anestrous ewes were studied in four experimental groups: (1) no progesterone, (2) 25 mg. progesterone per day for 6 days prior to ovulation, (3) 25 mg. progesterone per day for 6 days beginning on the expected day of ovulation and (4) 25 mg. progesterone per day for 12 days beginning 6 days prior to ovulation. The ewes were killed 6 days after the expected day of ovulation. The average corpus luteum weight and the number of ewes for these groups were, respectively: (1) 260.6 mg., 14 ewes; (2) 117.5 mg., 14 ewes; (3) 329.5 mg., 15 ewes; (4) 90.4 mg., 15 ewes. Progesterone injections beginning the expected day of ovulation did not reduce corpus luteum weight. Progesterone injections prior to the time of ovulation resulted in smaller (P<.01) corpora lutea whether or not the injections were continued after the time of ovulation.

**Literature Cited**


