INHIBITION BY PROGESTERONE OF IUD-INDUCED LUTEAL REGRESSION IN THE EWE

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Summary

OVULATION was induced on day 5 of an estrous cycle and each uterine horn was infected with *E. coli* or implanted with a plastic spiral on day 7. Both the original and the induced CL regressed in response to the infection. However, neither the original nor the induced CL regressed after the insertion of the IUD, even though 2-day-old CL are ordinarily susceptible to IUD-induced luteolysis. The results suggested that progesterone secretion by the original CL prevented IUD-induced regression of the new CL.

The injection of progesterone on days 1 to 4 of the estrous cycle prevented luteal regression following the insertion of an IUD on day 3. Also, the injection of progesterone on days 4 to 6 tended to counteract the enhancing effect of estradiol on CL regression after the insertion of an IUD on day 5. These results indicate that progesterone can prevent IUD-induced luteolysis in the ewe.

Introduction

Early regression of corpora lutea (CL) in the ewe can be induced by inserting an intrauterine device (IUD) into the uterine lumen within the first 3 days of the estrous cycle (Moore and Nalbandov, 1953; Inskeep *et al.*, 1962; Ginther, Pope and Casida, 1966). The insertion of an IUD after the fourth day of the cycle does not initiate luteal regression (Warren and Hawk, 1971).

Ovarian hormones influencing the ewe may determine whether the insertion of an IUD will cause luteolysis. The destruction of follicles by electrocautery delayed CL regression after the insertion of an IUD on the day after estrus (Ginther, 1971). Also, the administration of estradiol during the first few days of the estrous cycle extended the period of time that the insertion of an IUD would cause luteal regression (Warren and Hawk, 1971). The results of both of these studies suggest that estrogen may have a role in IUD-induced luteolysis. On the other hand, progesterone may interfere with IUD-induced CL regression. Bhalla and Casida (1970) induced ovulation in luteal phase ewes and inserted an IUD; they found that either the presence of the mature CL or the administration of progesterone prevented regression of the newly induced corpora lutea.

This study was conducted to acquire further information on the effect of ovarian hormones on IUD-induced luteal regression in the ewe.

Experimental Procedure

Three experiments were conducted with 95 ewes. Columbia ewes were used in experiment 1, and Rambouillets in experiments 2 and 3. Estrus was checked twice daily, using vasectomized rams. The first day of estrus was designated as day 0.

With each ewe, an IUD was inserted into the uterine lumen, an infection induced in the uterine lumen, or a sham operation performed on a uterine horn. On the designated day of an estrous cycle, the ewes were anesthetized with sodium pentobarbital and the uterus and ovaries exposed through a mid-ventral laparotomy. Corpora lutea were marked with sterilized India ink so that they could be identified at necropsy. Each IUD was a plastic spiral 9 mm in diameter and 30 mm long; it was inserted, as described previously (Warren and Hawk, 1971), into the lumen of a uterine horn adjacent to an ovary containing a corpus luteum. With sham-operated ewes, the IUD was inserted and removed immediately. Infection was induced by placing two ligatures about 10 cm apart around a uterine horn adjacent to a developing CL and inoculating the lumen of the ligated segment of horn with a suspension of *Escherichia coli* cells, as described previously (Brinsfield, Higginbotham and Hawk, 1969).
Since premature CL regression occurs more frequently on an ovary adjacent to an IUD-containing uterine horn than on the contralateral ovary (Ginther, Pope and Casida, 1966), only CL on the ovary adjacent to the IUD-containing or sham-operated horn are included in the results of experiments 2 and 3.

Progesterone or estradiol-17β were dissolved in corn oil and injected subcutaneously twice daily. Injection schedules are given with the results of each experiment.

At the time of necropsy, the CL were peeled from the ovarian stroma and classified as normal or regressed on the basis of their weight, color and firmness (Hawk and Bolt, 1970). The CL classified as regressing were small for the stage of the estrous cycle, and relatively pale and hard. In ewes with two CL of the same age (experiment 1), or in ewes with two CL on the ovary adjacent to a treated uterine horn (experiments 2 and 3), the average CL weight was used as the value for the ewe.

Weights of corpora lutea were compared among treatment groups by analysis of variance and Duncan’s Multiple Range test. For any two treatment groups, the statistical probability of a difference in the proportion of ewes having regressed CL was obtained from tables of exact probabilities for 2 x 2 contingency tests (McGuire, Lehmann and Heath, 1967).

**Results**

**Experiment 1. Stage of the Estrous Cycle as the Determining Factor in IUD-Induced Luteolysis.** Ovulation was induced in 12 ewes on day 5 of an estrous cycle with an intravenous injection of 750 IU of HCG in aqueous saline solution. On day 7, each uterine horn of individual ewes was sham-operated, implanted with an IUD, or infected with *E. coli* (table 1). Each ewe had one or two original CL and one or two induced CL. It was expected that the original CL, about 6 days old, would not be affected by the IUD; however, the induced CL were about 1 day old, an age that CL are ordinarily highly susceptible to the luteolytic effect of an IUD. The infection, a strong luteolytic stimulus if begun at any time before day 8 (Brinsfield et al., 1969), was used as a positive control treatment.

Both the original and the induced CL regressed in the infected sheep (table 1), demonstrating that CL of both ages were susceptible to regression by the presence of a strong irritant in the uterus. However, the IUD did not cause regression of either the original or the induced CL, suggesting that the stage of the estrous cycle and not the age of the CL determines whether a developing CL will regress when an IUD is placed in the uterus.

**Experiment 2. Prevention by Progesterone of IUD-Induced Luteal Regression.** On days 1 to 4 of an estrous cycle, 29 ewes were injected twice daily with 10 mg of progesterone in corn oil or with corn oil alone. An IUD was inserted or a sham-operation performed on day 3. The ewes were killed on day 9. Although exogenous progesterone alone injected on days 1 to 4 causes premature luteolysis, the CL do not usually regress until after day 9 (Ginther, 1968).

Corpora lutea were normal in weight, color and tone in each of six sham-operated control ewes and in six of seven sham-operated progesterone-injected ewes (Groups 1 and 3, table 2). The CL of progesterone-treated ewes (Group 3) were nonsignificantly (P>.05) lighter than those of the controls (Group 1). An IUD inserted on day 3 induced early CL regression in seven of eight corn oil injected ewes (Group 2), but only in two of eight IUD ewes injected with progesterone (Group 4, P<.05). This comparison, along with others on CL weights (footnote, table 2), indicates that the exogenous progesterone prevented IUD-induced luteal regression in most ewes.

**Experiment 3. An Attempt to Prevent the Estrogen Enhancement of IUD-Induced Luteolysis by Exogenous Progesterone.** Fifty-four ewes were injected twice daily with corn oil, progesterone, estradiol, or both hormones (table 3). Progesterone was administered at a higher level in this experiment than in experiment 2 because of the attempt to prevent the estrogenic effect. An IUD was inserted or a sham-operation performed on day 5. Ewes were killed on day 11.

The following treatments did not cause the

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**TABLE 1. ATTEMPTS TO CAUSE REGRESSION OF CORPORA LUTEA OF TWO AGES**

<table>
<thead>
<tr>
<th>Treatment of the uterus on day 7</th>
<th>No. of ewes</th>
<th>Weights of CL (mg±SE)</th>
<th>Original CL</th>
<th>Induced CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4</td>
<td>894±65</td>
<td>618±58</td>
<td></td>
</tr>
<tr>
<td>IUD</td>
<td>4</td>
<td>768±61</td>
<td>606±72</td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>4</td>
<td>207±70</td>
<td>64±11</td>
<td></td>
</tr>
</tbody>
</table>

*a* Corpora lutea were examined and weighed on day 14. Control or IUD or infected ewes, P<.001 for original or induced CL.

*b* Corpora lutea resulting from an intravenous injection of HCG on day 5.
INHIBITION OF LUTEAL REGRESSION

TABLE 2. PREVENTION OF IUD-INDUCED LUTEAL REGRESSION BY EXOGENOUS PROGESTERONE

<table>
<thead>
<tr>
<th>Treatment on days 1-4</th>
<th>Group</th>
<th>Total no. of ewes</th>
<th>Ewes with maintained CL</th>
<th>Ewes with regressed CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil, 1-6</td>
<td>4</td>
<td>1 4</td>
<td>4 (533±37) b</td>
<td></td>
</tr>
<tr>
<td>Progesterone, 3-6</td>
<td>3</td>
<td>6 (468±54)</td>
<td>1 (68)</td>
<td></td>
</tr>
</tbody>
</table>

a Interaction among the four groups for CL weights of the total No. of ewes, P<.01. Average luteal weight, corn oil-IUD vs. progesterone-IUD, P<.05.

b Mean CL weight (mg±SE). Corpora lutea were examined and weighed on day 9.

regression of CL in an appreciable proportion of ewes by day 11: The insertion of an IUD on day 5 (Group 2, table 3), progesterone alone (Group 3), progesterone in addition to an IUD (Group 4), estradiol alone (Group 5), or estradiol and progesterone (Group 7).

Estradiol, as expected, extended the stage of the cycle during which the insertion of an IUD caused luteolysis; CL were regressed in eight of 12 estradiol-IUD-treated ewes (Group 6). In Group 8, CL regressed in four of 12 ewes treated with progesterone in addition to estradiol and an IUD. Thus the administration of progesterone to estradiol-IUD treated ewes tended to reduce the proportion of ewes with regressed CL (Group 8 vs. Group 6, P=.10). The results were inconclusive, but suggested that in some ewes the progesterone may have counteracted the enhancement by estradiol of IUD-induced luteolysis. The duration of progesterone treatment may have been too short to prevent IUD-induced luteolysis in a higher proportion of ewes.

Discussion

The results of experiment 1 indicate that the stage of the estrous cycle is more important than the age of the CL in determining the luteolytic effect of an IUD. In this study, as well as in that of Bhalla and Casida (1970), 1 or 2 day old CL, which would ordinarily be susceptible to IUD-initiated luteolysis, did not regress in the presence of an older CL.

Progesterone apparently prevents the activation of a luteolytic mechanism by an IUD. The data from experiments 2 and 3 show that exogenous progesterone injected on days 1 through 4 of the cycle prevented the luteolytic effect of an IUD inserted on day 3, and that exogenous progesterone injected on days 4 through 6 tended to counteract the enhancing action of exogenous estradiol on IUD-induced CL regression. Bhalla and Casida (1970) found that a young CL developing after the induction of ovulation in a luteal phase ewe was susceptible to IUD-induced luteolysis if the original CL were removed; however, if the original CL were left intact or if exogenous progesterone was administered after the original CL were removed, the induced CL did not regress. Also, day 4 of the estrous cycle, when an IUD will no longer consistently cause CL regression (Warren and Hawk, 1971), coincides with an increasing level of endogenous progesterone secretion (Thorburn, Basset and Smith, 1969).

Bhalla and Casida (1970) reported that, in ewes treated with an IUD on day 2, the injection of progesterone for the first 8 days of the cycle did not prevent IUD-induced CL regression. In contrast, in ewes fitted with an IUD on day 3 (experiment 2), the injection of progesterone from day 1 to 4 generally prevented IUD-induced luteolysis. The greater effectiveness of the progesterone treatment in the present study may have been due

TABLE 3. INTERACTION OF EXOGENOUS PROGESTERONE AND ESTRADIOL ON IUD-INDUCED CORPUS LUTEUM REGRESSION

<table>
<thead>
<tr>
<th>Treatment and days of treatment</th>
<th>Group</th>
<th>Total no. of ewes</th>
<th>Ewes with maintained CL</th>
<th>Ewes with regressed CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil, 1-6</td>
<td>2</td>
<td>4</td>
<td>4 (519±32) b</td>
<td></td>
</tr>
<tr>
<td>Progesterone, 4-6</td>
<td>4</td>
<td>12</td>
<td>5 (544±23)</td>
<td>8 (91±42)</td>
</tr>
<tr>
<td>Estradiol, 1-6</td>
<td>5</td>
<td>12</td>
<td>5 (481±41)</td>
<td>8 (91±26)</td>
</tr>
<tr>
<td>Estradiol, 1-6, progesterone, 4-6</td>
<td>7</td>
<td>12</td>
<td>4 (514±35)</td>
<td>4 (51±4)</td>
</tr>
</tbody>
</table>

a For Groups 2, 3 and 4, the hormone injection schedules were as follows: Progesterone, 20 mg twice daily; estradiol, 80 µg twice daily. The hormones were injected subcutaneously in 1 ml of corn oil.

b Mean weight of CL (mg±SE). Corpora lutea were examined and weighed on day 11.
to the greater amounts of progesterone used or to the additional day of progesterone treatment before the IUD was inserted.

In agreement with earlier work (Warren and Hawk, 1971), the results of experiment 2 show that exogenous estradiol extends the period of the estrous cycle during which the insertion of an IUD will induce early CL regression. The exogenous estradiol presumably counteracted the influence of endogenous progesterone. It appears that an IUD can activate a luteolytic mechanism when the ewe is predominantly influenced by estrogen, or else when she is not strongly influenced by progesterone.

The activation of a luteolytic mechanism by an IUD must depend, at least initially, on some response of the uterus to the presence of the IUD. Also, the IUD-activated luteolytic mechanism operates at least partly through local utero-ovarian pathways (Ginther, Pope and Casida, 1966). Therefore, it is possible that injected progesterone and estradiol act on the uterus to influence the IUD-activated luteolytic mechanism.

Literature Cited


