EFFECT OF SUCCESSIVE ENUCLEATIONS OF BOVINE CORPORA LUTEA ON FORMATION AND FUNCTION OF SUBSEQUENT LUTEAL TISSUE

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It has been demonstrated that exogenous progesterone exerts a stimulatory effect on ovulation in cattle (Hansel and Trimberger, 1952) as well as an inhibitory effect (Ulberg, Christian and Casida, 1951; Trimberger and Hansel, 1955; Nellor and Cole, 1957; Labhsetwar et al., 1964). However, the role of endogenous luteal progesterone in the regulation of bovine corpus luteum formation and functional activity is conjectural.

The present study was conducted to determine the effect of endogenous luteal progesterone withdrawal, as achieved by successive enucleations of bovine corpora lutea, on (1) the formation of subsequent corpora lutea as determined by gross morphology and luteal tissue weight and (2) the functional activity of subsequent luteal tissue as estimated by initial progesterone concentration and de novo progesterone production during in vitro incubation.

Materials and Methods

Corpora lutea were obtained from four nulliparous Holstein heifers (body weight: 365 to 470 kg.) following the establishment of at least one normal estrous cycle for each animal. Corpora lutea were enucleated from each heifer on day 10 postestrus of four successive estrous cycles via a supravaginal incision. Since the supravaginal incision was not sutured, each successive gland was removed through the initial incision which was reopened by slight pressure of the forefinger. A fifth gland was obtained from each heifer at time of slaughter which was scheduled for day 10 postestrus following the fourth enucleation or 14 days after the fourth enucleation if the animal failed to return to estrus. Luteal tissue removed after slaughter, however, was not analyzed for progesterone due to the extended interval from recovery of the gland at slaughter until the gland could be returned to the laboratory for analysis. Each gland removed via the supravaginal incision was immediately cooled on iced, saline-moistened toweling; dissected free of adhering connective tissue, grossly examined and weighed. Luteal tissue was sliced (0.3 mm. maximum thickness) and samples (200 mg.) were placed in 5 ml. of Krebs-Ringer bicarbonate buffer containing 2 mg./ml. glucose. Samples were incubated for 2 hr. at 37–38°C. under a 95% O2: 5% CO2 atmosphere. The interval from enucleation to the beginning of incubation ranged from 16 to 75 min. (Mean: 50 min.). Cold 95% ethanol was added to unincubated samples to stop metabolic activity. Unincubated and incubated samples and their respective media were stored in 95% ethanol at −15°C. until analyzed for progesterone by a modification of the method described by Stormshak et al. (1963). The method used involved extraction with 95% ethanol, silica gel column and paper chromatography and final quantitation by ultra-violet spectrophotometry. Preliminary analyses showed that the elimination of the solvent partitioning phase originally described by Stormshak et al. (1963) resulted in no significant reduction in purification efficiency. Corrections for progesterone losses during purification were made on the basis of recovery rates of trace amounts of progesterone-4-C14 added to each sample prior to initial ethanolic extraction (Mean recovery rate: 76.2%).

Triplicate determinations were made on all corpora lutea and the average of the two numerically closest values was used as the estimate of progesterone concentration (mcg./gm. of fresh luteal tissue). De novo progesterone production represented the progesterone concentration of incubated samples minus the progesterone concentration of unincubated samples. Data were statistically analyzed by the analysis of variance (Steel and Torrie, 1960).
Results

Control estrous cycles for the four heifers prior to enucleation of the first corpus luteum averaged 21 days (range: 19 to 23 days). The intervals from enucleation at day 10 post-estrus to return to estrus ranged from 2 to 7 days (Mean: 4 days) with the exceptions of a 26-day interval for Heifer 1 following the first enucleation and failures of Heifers 2 and 3 to return to estrus within a 14-day period from the fourth enucleation to the time of slaughter. The 26-day interval for Heifer 1 is believed to be associated with the failure to completely remove all luteal tissue when the CL ruptured during the first enucleation. In this case, it is possible that the extended interval represents a 4 or 5 day postoperative period to an undetected ovulation followed by a normal 21 day interval to a second ovulation. In general, repeated enucleation caused no change in the return interval until after the fourth enucleation when two of the four animals failed to return to estrus within a 14-day period. Intensity and duration of estrus, although not measured directly, were not apparently affected by successive enucleation. In all cases, heifers recovered rapidly from surgery and showed no signs of generalized infection due to repeated opening of the supravaginal incision. However, after the third or fourth enucleation, fibrinous adhesions began to develop around one or both ovaries of the experimental animals.

Formation and Weight of Consecutive Ovarian Structures. Successive enucleations of luteal tissue at day 10 postestrus resulted generally in the formation of increasingly more cystic structures, progressing from normal corpora lutea (N–CL) to cystic corpora lutea (C–CL) with fluid-filled central cavities to large luteinized follicles (L-fol) (table 1). A structure was considered to be a cystic corpus luteum if it contained a fluid-filled central cavity of 7 mm. or more in diameter and possessed an ovulation papilla. A luteinized follicle was a structure which contained a fluid-filled central cavity of 16 mm. or more in diameter surrounded by a wall of luteinized tissue of at least 2 mm. in thickness, but did not possess an apparent ovulation papilla. Exceptions to the formation of increasingly more aberrant structures with successive enucleations were the development of normal corpora lutea by Heifers 2 and 3 following the removal of the fourth and third ovarian structure, respectively.

As indicated in table 1, twin luteinized structures were obtained from Heifer 2 at the third enucleation and from Heifer 4 following slaughter. At the time of slaughter, a large follicle (15 to 18 mm. in diameter), in addition to a luteinized structure, was recovered from both Heifers 2 and 3. In all other cases, only a single significant ovarian structure was detected at the time of enucleation on day 10 postestrus.

Luteal tissue weights (exclusive of cystic fluid) for the luteinized structures are shown in table 1. As indicated in table 1, there was a failure to completely enucleate the first gland from Heifer 1, therefore, the weight of the tissue obtained was not included in the statistical analysis of luteal tissue weight. Analysis of variance of these data showed no significant differences (P > .05) between mean luteal tissue weights of the five consecutive ovarian structures. Similarly, there were no significant between-heifer differences for mean luteal tissue weights.

Functional Activity of Consecutive Luteal Tissue. Initial progesterone concentrations (mcg./gm. of fresh tissue) and de novo progesterone production during in vitro incubation (mcg./gm./2 hr.) of consecutive luteal tissues are shown in table 2. The mean initial progesterone concentration of corpora lutea obtained at the first enucleation was somewhat lower than the mean values for glands obtained at subsequent enucleations; however, differences between mean values for the four groups of consecutively enucleated luteal tissue did not prove to be significant (P > .05). Between-heifer differences in mean initial progesterone concentration also were not statistically significant (P > .05).

Mean de novo progesterone production values for luteal tissue obtained at the second and third enucleations were slightly lower than the mean values for glands removed at the first and fourth enucleations. Analysis of variance showed no significant differences between mean de novo progesterone production values for consecutive luteal tissue. Between-heifer differences in mean de novo progesterone production, however, were significant (P < .01), indicating considerable individual variability in steroidogenic capacity of luteal tissue from different animals. Initial progesterone concentration and de novo progesterone production for consecutive luteal tissue suggest that successive enucleations had no significant effect on the functional activity of subsequent luteal tissue regardless of the formation of progressively more aberrant structures.
<table>
<thead>
<tr>
<th>Heifer no.</th>
<th>Consecutive ovarian structures a</th>
<th>Luteal tissue wt. (gm.)</th>
<th>Consecutive ovarian structures a</th>
<th>Luteal tissue wt. (gm.)</th>
<th>Consecutive ovarian structures a</th>
<th>Luteal tissue wt. (gm.)</th>
<th>Consecutive ovarian structures a</th>
<th>Luteal tissue wt. (gm.)</th>
<th>Mean Luteal tissue wt. (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N-CL 0.35 a</td>
<td>C-CL 5.39</td>
<td>C-CL 6.05</td>
<td>L-fol 5.48</td>
<td>C-CL 5.44</td>
<td>5.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>N-CL 6.53</td>
<td>C-CL 7.52</td>
<td>L-fol 7.54 a</td>
<td>L-fol 5.83</td>
<td>N-CL 4.12</td>
<td>6.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C-CL 6.63</td>
<td>C-CL 4.89</td>
<td>L-fol 6.96</td>
<td>N-CL 5.79</td>
<td>L-fol 5.91</td>
<td>5.91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>N-CL 4.90</td>
<td>N-CL 5.09</td>
<td>C-CL 9.35</td>
<td>L-fol 6.67</td>
<td>L-fol 5.87 a</td>
<td>6.38</td>
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<tr>
<td>Mean</td>
<td>6.02</td>
<td>5.72</td>
<td>7.48</td>
<td>5.94</td>
<td>5.18</td>
<td>6.07</td>
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</table>

a N-CL: Normal corpus luteum.
C-CL: Cystic corpus luteum, containing a fluid-filled cavity 7 mm. or more in diameter and having an apparent ovulation papilla.
L-fol: Luteinized follicle, containing a fluid-filled cavity 16 mm. or more in diameter and having no apparent ovulation papilla.
NL-fol: Non-luteinized large follicle; over-all diameter 10 to 20 mm.
b Ovarian structures removed after slaughter.
c Incomplete enucleation of luteal tissue; value not included in means or in analysis of data.
d Value represents the combined luteal tissue weight of twin structures.
FORMATION AND FUNCTION OF LUTEAL TISSUE

TABLE 2. EFFECT OF SUCCESSIVE CORPUS LUTEUM ENUCLEATIONS ON INITIAL PROGESTERONE CONCENTRATION AND DE NOVO PROGESTERONE PRODUCTION OF SUBSEQUENT LUTEAL TISSUE

<table>
<thead>
<tr>
<th>Heifer no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Mean initial</th>
<th>Mean de novo</th>
</tr>
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<tr>
<td>1</td>
<td>49.0</td>
<td>55.2</td>
<td>96.0</td>
<td>23.6</td>
<td>43.0</td>
<td>33.4</td>
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<td>2</td>
<td>25.3</td>
<td>85.6</td>
<td>53.6</td>
<td>62.9</td>
<td>58.5</td>
<td>49.6</td>
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<tr>
<td>3</td>
<td>21.9</td>
<td>29.4</td>
<td>37.4</td>
<td>33.8</td>
<td>62.3</td>
<td>28.7</td>
</tr>
<tr>
<td>4</td>
<td>38.6</td>
<td>42.8</td>
<td>31.8</td>
<td>35.2</td>
<td>74.4</td>
<td>13.1</td>
</tr>
<tr>
<td>Mean initial</td>
<td>33.7</td>
<td>54.7</td>
<td>59.6</td>
<td>53.2</td>
<td>54.9</td>
<td>38.9</td>
</tr>
</tbody>
</table>

*Value represents the mean of two structures.*

Discussion

Observations in this study are not in complete agreement with the report of Foote et al. (1959) who observed a tendency for dairy heifers to develop cystic follicles or to produce twin ovulations following the removal of only one or two corpora lutea at day 14 postestrus. However, Foote et al. (1959) did not report whether or not the cystic structure contained luteal tissue. In the present study the removal of one or two corpora lutea generally resulted in the formation of a cystic corpus luteum in the subsequent cycle. A marked increase in the development of twin ovarian structures (luteinized and large follicles) did not occur until after the fourth enucleation. Associated with the development of twin ovarian structures following the fourth enucleation was the failure of two of the four heifers to show signs of estrus within the expected 2- to 7-day return interval. It is suggested that the stage of the estrous cycle at which the corpus luteum is removed may contribute to the type of ovarian structure subsequently formed.

The present study shows that endogenous progesterone withdrawal, as achieved by successive luteal tissue enucleations at day 10 postestrus, results in the formation of increasingly more aberrant ovarian structures progressing from cystic corpora lutea to luteinized follicles which failed to ovulate. However, successive enucleations had no significant effect on either the amount of luteal tissue formed or the functional activity of subsequent luteal tissue as estimated by initial progesterone concentration and de novo progesterone production during in vitro incubation.

Based on cytological studies of the degranulation of delta (gonadotropin) cells in the adenohypophyses of cows with normal and cystic ovaries, McEntee and Jubb (1957) postulated that luteal cysts form when insufficient luteinizing hormone (LH) is released and that cystic follicles develop when even less LH is released. Spies et al. (1959) produced cystic corpora lutea in gilts with progesterone injections and suggested, as did Foote et al. (1958), that cyst formation is due to LH inhibition.

Accordingly, it is suggested that the present results may be explained on the basis of a progressive depletion of pituitary LH reserves as the result of the removal of progesterone inhibition of pituitary LH release. Cystic corpora lutea and eventual luteinized follicles associated with ovulation failure are formed due to a lack of an acute discharge of sufficient LH to initiate ovulation and normal CL formation. Nevertheless, it appears that LH secretion is sufficient to stimulate the formation of relatively normal amounts of luteal tissue and maintain its functional activity within normal limits.

Assays of pituitary and serum gonadotropins will be necessary to further elucidate the effect of progesterone withdrawal, as achieved by successive luteal tissue enucleations, on subsequent corpus luteum formation and function.

Summary

Four nulliparous Holstein heifers were used in an experiment to determine the effect of endogenous luteal progesterone withdrawal, as achieved by successive enucleations of luteal tissue, on the formation and functional activity of subsequent luteal tissue. Glands were enucleated from each heifer at four consecutive times on day 10 postestrus via a supravaginal incision.

Successive enucleation of luteal tissue at day 10 postestrus resulted generally in the formation of increasingly more cystic ovarian structures, progressing from normal corpora
lutea to cystic corpora lutea to luteinized follicles associated with ovulation failure. However, the consecutive removal of luteal tissue had no significant effect on either the amount of luteal tissue formed or the functional activity of subsequent luteal tissue. A mechanism for the explanation of these results is discussed.

**Literature Cited**


