THE effects of exogenous progesterone on the corpus luteum are quite variable. No gross or microscopic effects were apparent in the pregnant rat (Sammelwitz et al., 1956; Aldred et al., 1959) or rabbit (Ulberg, 1952). Selye (1939) reported no effect of progesterone on corpora lutea of late pregnancy in mice, but Burdick (1942) found that treatment begun one or two days post-mating caused luteal regression.

Whether or not progesterone is detrimental to corpora lutea, and the extent to which the corpora lutea are damaged, appears to depend upon the time of injection in gilts (Sammelwitz and Nalbandov, 1958) and ewes (Zimbelman et al., 1959). Spies et al. (1958) found that progesterone treatment from the 10th to the 25th day after mating caused regression of corpora lutea in hysterectomized as well as in pregnant gilts, indicating that progesterone acts independently of the uterus.

Estrogen treatment has a beneficial effect on corpora lutea in rats (Hohlweg, 1934; Desclin, 1935; Selye et al., 1935; Wolfe, 1935; Merckel and Nelson, 1940) and rabbits (Allen and Heckel, 1936; Heckel and Allen, 1939). Kidder et al. (1955) found that stilbestrol caused luteinization of follicles when injected on day-11 of the estrual cycle of gilts. Hammond and Day (1944) observed that corpora lutea persisted for prolonged periods in heifers implanted with stilbestrol, while Greenstein et al. (1958) reported that estradiol administered from day-2 through day-12 of the estrual cycle caused early regression of corpora lutea.

The purpose of this study was to determine the effects of injected progesterone and estrogen on the progesterone content, proportion of functional luteal cells, and weight of corpora lutea in open heifers.

Materials and Methods

The animals selected for these studies were observed through at least one estrual cycle and only those were used whose cycles were in the range of 17–25 days. The experiments were carried out so that each animal served as her own control. For this purpose, a “control” corpus luteum was removed via supra-vaginal incision on day-14 of an untreated estrual
cycle. An interval of at least one ovarian cycle elapsed between the time that the control corpus was removed and the experimental treatment was begun. Three groups of 10 animals each were used in this investigation. One group (composed of one virgin heifer, one multiparous, and eight uniparous cows) was used to study the effects of progesterone injected on day-1 of the estrual cycle. Another group (all virgin heifers) was used in a study of effects of progesterone injected 5 days after estrus, and still another group of virgin heifers was used to determine the effects of estrogen treatment from day-1 through day-13 of the estrual cycle. Progesterone-treated animals were given a single subcutaneous injection of one mg. of progesterone in starch suspension per lb. body weight on the appropriate day of the treated estrual cycle. Heifers on estrogen treatment received 250 mcg. of estradiol-17β in corn oil per day subcutaneously on day-1 through day-13 of the treated estrual cycle. Corpora lutea were removed either at slaughter or by supra-vaginal incision on day-14 of the cycle.

Corpora lutea were weighed fresh and a small piece removed and fixed in Bouin’s solution for histological study. The remainder of the gland was stored frozen in 95% ethanol and assayed chemically for progesterone at a later time. Sections of corpora lutea were stained with hematoxylin and eosin and characterized according to the percentage of type I and II luteal cells (Foley and Greenstein, 1958), which were the types presumed to be “functional”. These percentages were transformed to angles for statistical analysis. Chemical assays for progesterone were usually done in duplicate by a modification of the method of Loy et al. (1957). The modification consisted of substituting for the hot 95% ethanol extraction, a similar extraction carried out at room temperature on a rotary shaker, and altering the proportions of chloroform used to develop and elute the column from 5% to 10% and from 20% to 25%.

In earlier work, extracts of swine corpora lutea were found to contain only progesterone (Loy et al., 1957). However, Gorski et al. (1957) reported finding Δ4-pregnen-20β-ol-3-one in extracts of bovine ovaries, and Hayano et al. (1954) reported this compound to be produced when progesterone was incubated with bovine corpus luteum tissue. It seemed advisable to examine extracts obtained in this study for the presence of such a substance. Twelve such extracts were chromatographed on paper in either a methylcyclohexane-propylene glycol or 80% methanol-Skellysolve B system. A contaminating steroid, thought to be Δ4-pregnen-20β-ol-3-one on the basis of chromatographic behavior was found, but the mean level was less than 5% of the total steroid.

The finding of a steroid not progesterone in the purified extract of bovine corpora lutea, plus the fact that levels of steroid found in this study and others from this laboratory are somewhat higher than those found by Gorski et al. (1958), and several times higher than those found
by Melampy et al. (1959), made it appear desirable to establish more
certainly that the major steroid component isolated was progesterone.

In comparisons of biological activity on rabbit uteri (Allen, 1930) with
chemical assay, crude extracts in amounts equivalent to 560 and 765 mcg.
of progesterone, as determined by chemical assay on comparable purified
aliquots, gave reactions of ++ to ++++, and that equivalent to
1040 mcg. gave a +++ reaction. Highly purified extracts eluted from
paper chromatograms were tested biologically on three rabbits in amounts
chemically estimated, directly from these extracts, to be equivalent to 500,
1000, and 1000 mcg. of progesterone. Responses to these extracts were ++
to ++++, ++++ to ++++, and ++++, respectively. Thus tests of
biological activity agree well with chemical assays.

Steroid purified by countercurrent distribution after paper chromato-
tography was crystallized from aqueous methanol. The spectrum of its
sulfuric acid chromogen between 220 and 500 m\u00b4 exhibited a single
absorption peak at 293 m\u00b4 which is the approximate wave length at which
progesterone shows a similar maximum (Bernstein and Lenhard, 1953).
The infrared spectrum of the crystallized material showed the same
absorption bands as the reference spectrum of \(\beta\)-progesterone and that
published for progesterone (Dobriner et al., 1953) in the spectral range
of from approximately 700 to 1850 cm\(^{-1}\). These results indicate that the
compound which was isolated as the major steroid component is proges-
terone. Since the substance present as contaminant is assumed to be a
naturally occurring progestogen (Zander et al., 1958), the values used
for statistical comparisons are those for progesterone and this substance
combined.

Comparisons between treated and control values were made by the
Student t-test or by the H-test of Kruskal and Wallis (1952). Correlations
were calculated within each control and treated group. These were not
heterogeneous for the three control groups, so sums of squares and cross
products were pooled for calculation of correlations within the control
groups.

Results

Progesterone injected on the day after estrus exerted a detrimental effect
on corpora lutea as indicated by significant differences between treated
and control for all three characteristics studied (table 1). The individual
response of corpus luteum weight and proportion of functional luteal cells
to treatment on day-1 was quite variable as shown by significantly in-
creased variances. This tended to be true for progestogen concentration
also, but the increase in variance was not significant.

Treatment with progesterone 5 days after estrus reduced progestogen
concentration and percentage of functional cells significantly. It had no

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\(^3\) Infrared analysis was done by Dr. Alan F. Krivis of the Upjohn Company, Kalamazoo, Michigan.
TABLE 1. MEANS AND VARIANCES OF CHARACTERISTICS OF CORPORA LUTEA FROM OVARIAN HORMONE-TREATED HEIFERS AND OF CONTROL CORPORA LUTEA

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Progesterone, day-1</th>
<th>Progesterone, day-5</th>
<th>Estrogen, days 1-13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>Corpus luteum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Av. wt., gm.</td>
<td>5.17</td>
<td>3.63**</td>
<td>4.34</td>
</tr>
<tr>
<td>Variance</td>
<td>1.22</td>
<td>5.67*</td>
<td>1.62</td>
</tr>
<tr>
<td>Progestogen conc.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Av., mcg./gm.</td>
<td>49</td>
<td>29*</td>
<td>46</td>
</tr>
<tr>
<td>Variance</td>
<td>232</td>
<td>405</td>
<td>97</td>
</tr>
<tr>
<td>Functional luteal cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Av., %</td>
<td>86</td>
<td>36**</td>
<td>85</td>
</tr>
<tr>
<td>Variance</td>
<td>23</td>
<td>786**</td>
<td>21</td>
</tr>
</tbody>
</table>

* Differs from control, P<0.05.
** Differs from control, P<0.01.

significant effect on the weight of corpora lutea, however, and even the downward trend was slight (table 1). Only the variance of percentage of functional cells was increased significantly by progesterone on day-5.

Administration of estradiol for 13 days beginning on the day after estrus exerted a marked depressing effect on the corpus luteum weight and percent of functional luteal cells, but progestogen concentration was not significantly changed by this treatment. Estrogen injection tended to increase the variance of all characteristics but the increase was significant only for the percentage of functional luteal cells (table 1).

The effect of progesterone treatment on day-1 is reflected also by the positive associations between weight, proportion of functional cells and progestogen concentration, which are significantly different from those in control corpora lutea (table 2). No significant changes in these associations

TABLE 2. CORRELATIONS AMONG CHARACTERISTICS OF CORPORA LUTEA FROM OVARIAN HORMONE-TREATED HEIFERS AND OF CONTROL CORPORA LUTEA

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Progesterone</th>
<th>Estrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day-1</td>
<td>day-5</td>
</tr>
<tr>
<td>Variables correlated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells and functional weight</td>
<td>0.46*</td>
<td>0.27</td>
</tr>
<tr>
<td>Conc. and weight</td>
<td>0.65**</td>
<td>——.36</td>
</tr>
<tr>
<td>Conc. and functional cells</td>
<td>0.79**</td>
<td>0.51*</td>
</tr>
</tbody>
</table>

* Differs from control, P<0.05.
** Differs from control, P<0.01.
* Differs from control, P<0.10.
* Correlation significant, P<0.05.
** Correlation significant, P<0.01.
were effected by progesterone treatment on day-5. Associations between corpus luteum weight and percentage of functional cells and between weight and progestogen concentration were significantly changed by estrogen treatment, but that between functional cells and concentration was not (table 2).  

**Discussion**  
The results obtained by the injection of ovarian hormones appear to be explainable on the assumption that formation of the corpus luteum and maintenance of its function are dependent upon optimal ratios of estrogen and progesterone. The mechanisms by which imbalances of the ovarian hormones damage the corpus luteum are presumed to involve altered pituitary gonadotrophin secretion.

It has been shown that certain dosages of progesterone will prevent heat and ovulation and yet not interfere with follicular development (Dutt and Casida, 1948; O'Mary et al., 1950; Ulberg et al., 1951a, 1951b). Foote et al. (1958) found significantly higher levels of LH in the pituitary glands of gilts slaughtered immediately following a period of progesterone treatment than in those of similar gilts slaughtered 8 days later. It may thus be postulated that altering the ratio of ovarian hormones by injecting progesterone tends to depress LH activity by inhibiting its release from the pituitary; treatment following ovulation consequently would result in formation of corpora lutea which are poorly developed and maintained.

The regressive histological changes and lowered progestogen content with normal weight of corpora lutea from animals injected on day-5 suggest impaired maintenance. The relatively greater effects on day-1 are presumed to result both from faulty formation because of an early deficiency of LH, and from regressive changes due to lowered maintenance. Progesterone injection at comparable stages of the estrual cycle in swine (Sammelwitz and Nalbandov, 1958) failed to bring about reduction of corpus luteum weight; however, studies of other criteria of function of the corpora lutea were not reported.

Estrogen treatment causes production and/or release of LH, resulting in luteinization in rats (Hohlweg, 1934; Desclin, 1935; Wolfe, 1935; Merckel and Nelson, 1940) and swine (Kidder, 1955), although Kidder (1954) was unable to demonstrate this in the cow with the relatively large doses that were tested. It is postulated that stored LH is released first by estrogen treatment, following which that hormone is released as rapidly as it is produced (Fevold et al., 1936; Hellbaum and Greep, 1946). Thus the luteinizing activity following the initial release may be quite low if treatment is continued. Corpus luteum formation may be limited by early depletion of the LH supply, and regressive histological changes may result from inadequate amounts of luteinizing hormone following formation.

An alternative explanation for the degenerative changes in corpora lutea resulting from estrogen treatment is that the LH being continually released
in small amounts following formation of corpora may become luteolytic (Greep, 1938). This view would not be consistent with the interpretation of results of progesterone treatment on day-5 as being due to the blocking of LH release and a consequent impairment of maintenance. The possibility exists that the effects of injected ovarian hormones on the corpus luteum are mediated by way of a luteotrophic substance other than LH, for example, lactogen, but the effectiveness of such a principle has not been demonstrated in tests on the bovine (Smith et al., 1957).

Apparent differences between cattle and swine in the effects of injected ovarian hormones on the corpus luteum require further study. Some evidence suggests that the action of injected progesterone in preventing the release of LH from the pituitary glands of these species is similar (Ulberg et al., 1951a, 1951b). The release of LH by estrogen has not been demonstrated in cattle. The effect in swine may not be uniform under all conditions (Kidder et al., 1955; Foote et al., 1958), and a similar situation may exist for the cow. Just what ratio of estrogen to progesterone could be expected to favor release of hypophyseal LH is not known. Further investigations should be made to ascertain whether or not estrogen causes release of LH in cattle, and, if so, under what conditions of dosage level and duration and at what stages of the estrual cycle or in the presence of what progesterone levels this might occur.

Summary

The effects of injected ovarian hormones on the corpus luteum of the estrual cycle were studied in heifers. One mg. of progesterone per lb. body weight in a single injection on day-1 of the cycle caused significant differences in corpus luteum weight, proportion of functional luteal cells, and progestogen concentration between treated and control corpora lutea on day-14. Positive associations between functional cells and weight, concentration and weight, and concentration and functional cells (r=0.46, 0.65, and 0.79, respectively) were significantly different from corresponding control correlations (r=−.22, −.10, and −.07, respectively). The same hormonal treatment on day-5 of the estrual cycle caused significant differences in proportion of functional cells and progestogen concentration, but not in corpus luteum weight. No significant change in associations of these characteristics was caused by this treatment. When heifers were given 250 mcg. of estradiol-17β per day from day-1 through day-13 of the estrual cycle, significant differences were found between treated and control corpora lutea for weight and proportion of functional luteal cells, but not for progestogen concentration. Associations between functional cells and weight, and concentration and weight were significantly altered from control values (r of 0.52 and 0.85 vs. −.22 and −.10, respectively), but the correlation between concentration and functional cells was not significantly affected by treatment (0.55 vs. −.07).
Literature Cited


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