PROGESTINS IN BOVINE CORPORA LUTEA, OVARIES, AND ADRENALS DURING PREGNANCY

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SUMMARY

Progesterone and Δ⁴-pregnenec-20 β-ol-3-one (20 β-ol) were assayed in 89 corpora lutea and 54 pairs of ovaries (corpora lutea removed) and adrenals collected from cows pregnant 16 to 275 days. Levels of the two progestins were significantly higher in corpora lutea from 16 to 89 days (33.6 μg/g) than at 90 to 179 (20.4 μg/g) or after 180 days (27.5 μg/g) of pregnancy. Progesterone per gram of luteal tissue was significantly less at 90 to 179 days (12 μg/g) than after 180 days (18.7 μg/g) or for 16 to 89 days (20.4 μg/g). Progesterone and 20 β-ol per gram of luteal tissue were not highly correlated at any stage of pregnancy and the correlation for the study was only 0.21. There was a significant correlation (51 comparisons) between weight of the corpus luteum and ovary weight (r = 0.45) and adrenal (r = 0.30). Corpora lutea were not significantly heavier (P<0.10) at 16 to 89 days (5.9 g.) as compared with 90 to 179 days (5.0 g.) or after 180 days (5.5 g.). The progesterone content of the ovaries and adrenals was generally less than (1 μg/g) and did not vary significantly during pregnancy. The 20 β-ol level was very low in ovaries and this compound was not positively identified in adrenals. This study suggests that the corpus luteum remains functional throughout pregnancy in the bovine.

Prior to the identification of progesterone as the active component of the corpus luteum, Corner and Allen (6) reported that this structure is essential to the maintenance of pregnancy in the rabbit. Similar conclusions have been made from work with rats (15, 16, 39) and goats (21). Experiments conducted with other placentalia have shown that the corpus luteum is essential only during early pregnancy. Removal of the corpus luteum after the 35th day in humans does not terminate pregnancy (32). Studies with several other species such as the monkey (14), guinea pig (18), horse (13), and ewe (5) have shown results similar to those for the human (32).

Studies with the cow are inconclusive, though it appears fairly certain that an active corpus luteum is required at least during the first 200 days of pregnancy. Wester (33) found that ovariectomy of pregnant cows causes abortion. Sartoris (26, 27) terminated pregnancy in cows by ovariectomy during the sixth month. Raeside and Turner (23) gave daily subeutaneous injections of 25 mg. of progesterone, starting one day prior to the removal of the corpus luteum from heifers pregnant 44, 48, and 76 days. Abortion occurred in all three cases.

More recently, McDonald et al. (20) removed the corpus luteum from cows pregnant 207 to 236 days. Pregnancy terminated 50, 3, 24, 18, and 8 days, respectively, when the corpus luteum was removed at 207, 221, 224, 230, and 236 days.

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days of pregnancy. Erb (7) has observed that removal of the ovary containing the corpus luteum from three cows pregnant from 251-254 days resulted in abortion within 48-72 hr. Results have varied, depending on whether the corpus luteum or the entire ovary bearing it is removed. However, it seems clear that in the cow the corpus luteum is required for at least the first 8 mo. of pregnancy. Then, the essentiality of the corpus luteum of pregnancy in cows is similar to rabbits, rats, and goats, and quite different from primates, horses, and sheep.

The differences among species in regard to pregnancy maintenance following removal of luteal tissue prompted early investigators to suggest extra-ovarian sources of progesterone. Adler et al. (1), using the proliferation of the endometrium of the rabbit as positive evidence, reported that bovine and human placentae were sources of progestins. In 1952, Salhanick et al. (25) reported the initial isolation of progesterone from the human placenta. Since then, others have confirmed this finding (28, 37). Short (28, 30) has isolated progesterone from placenta of the mare, but could not detect this hormone in the late-term placentae of the cow, ewe, sow, or bitch. Gorski et al. (12) have reported similar results for bovine placentae.

In contrast to these results, Melampy et al. (22) recently reported the detection of progesterone in placenta, allantoic and amniotic fluids of the bovine. The amniotic fluid of the monkey has been found to contain measurable quantities of progestins (9). Forbes (8) has collected blood from the umbilical artery and veins of human and goat feti just before their separation from the placenta. He found that levels of progesterone in blood plasma from the umbilical artery were significantly higher than those from the veins.

Both Δ₄-pregnene-20 β-ol-3-one (20 β-ol) and Δ₄-pregnene-20 α-ol-3-one (20 α-ol) have been isolated and identified from the placental extracts of the mare (29) and human (24, 29, 37), but Wiest (34) could not detect these metabolites in extracts of rat placenta.

In 1938, Beall (4) isolated and identified progesterone and 3:20 allopregnanolone in extracts of the bovine adrenals. The adrenal gland has been suggested as a possible source of progestational activity during gestation. Balfour et al. (2) measured progesterone levels in adrenal venous blood of cows pregnant 210 and 240 days, respectively. They obtained values of 46.8 and 30.8 μg/100 ml. of plasma. They found that progesterone concentration of adrenal venous blood plasma was ten to 100 times greater than the concentration of progesterone in arterial blood at the same time. Progesterone was also detected in the adrenal glands of the sheep and the pig. Balfour et al. (3) have recently reported the isolation and identification of 20 α-ol from the effluent adrenal blood of young calves. Lombardo and Hudson (19) studied the in vitro biosynthesis of adrenocortical hormones by the human adrenal gland. When 3β-hydroxy-Δ₄-pregnene-20-one was added to the substrate, progesterone and 17 β-hydroxyprogesterone were isolated.

From the data pertaining to progestin levels in the bovine, it currently appears that: (a) Progesterone and 20 β-ol are the major progestational com-
pounds in ovarian tissue; (b) the adrenal glands are significant sources; and (c) the placenta is a questionable source.

The purpose of the present work was to investigate the levels of progestins in corpora lutea, ovaries, and adrenals of cows in various stages of pregnancy.

MATERIALS AND METHODS

Ovaries and adrenals of pregnant cows were removed at the time of slaughter. The corpus luteum was removed from the ovary bearing it and the tissues were separately frozen in solid CO₂ and stored at −25 °C until prepared for assay. Stage of pregnancy was determined from breeding dates or from the crown-rump measurements of the fetus as described by Winters et al. (35). The latter procedure was used for 28 cows pregnant from 105 to 275 days. Of 89 corpora lutea from pregnant cows, 29 were from Kansas State University. Ovaries and adrenals were not available for this group. Six samples of ovaries and adrenals were lost during assay, owing to contamination of the samples, leaving data on 54 pairs of ovaries and adrenals.

The reproductive tracts of all animals were examined to observe the condition of the embryo or fetus and to detect gross abnormalities. In cases of early pregnancy, the uterus, uterine horns, and Fallopian tubes were flushed with distilled water and the contents examined under a wide-field dissecting microscope.

Progesterone and 20 β-ol were separately determined in the corpus luteum and the combined ovaries and adrenals of individual cows, using the method of Stormshak et al. (31).

RESULTS AND DISCUSSION

General observations. The data were initially surveyed by grouping the samples according to month of gestation. Figure 1 shows that the changes in the average weight of corpora lutea, ovaries, and adrenals were not very great from 1 through 8 mo. of gestation, though on an average they were slightly heavier during the first 16–89 days. However, progesterone and 20 β-ol per gram of tissue, and particularly the latter, were noticeably higher during the first 3 mo. as compared with later stages of gestation. Average progesterone/gram of luteal tissue for 14 cows in the 210- to 239-day grouping averaged nearly as high (19.6 μg/g) as ten cows in the 30- to 59-day period (22.4 μg/g). However, two cows in the 210- to 239-day group had concentrations of progesterone higher than any of the other cows in the study.

Adrenal glands were heavier on the average at 30 to 59 days, 120 to 149 days, and 180 to 209 days. One cow in each group was primarily responsible for this, since their adrenal glands were nearly twice as heavy as the study average.

Since the groupings by month of gestation had too few data per month (range 6 to 14/group) and the cow-to-cow variation was considerable, the pregnancy periods were grouped into three successive 90-day intervals. Since cows of several breeds were involved, a check was made for possible breed differences.
There was little difference between breeds with respect to average levels of progesterone and 20 β-ol per gram of luteal tissue within the three gestation-stage groupings.

The average size of the corpus luteum did show some breed-to-breed variation which was not significant at P < 0.10. The corpora lutea of 15 Guernseys averaged the heaviest (6.8 g.) and 16 Herefords averaged the least (4.5 g.). The corpus luteum of cows pregnant 16-89 days averaged 5.9 g., which was heavier than for cows pregnant 90-179 days (P < 0.10) or over 180 days (P < 0.25). Though corpora lutea of Jersey, Guernsey, and Ayrshire cows were heavier during the 16- to 89-day stage of pregnancy as compared with the two later stages, Holsteins and Herefords were not. Therefore, it is questionable that any real difference exists in the size of the corpus luteum during
the three stages of pregnancy. Breeds were not considered further in the following summaries.

**Total quantities of progestins in corpora lutea.** Table 1 shows that progesterone, 20 ϒ-ol, and the two combined averaged considerably higher at 16-89 days than during the later stages of pregnancy (P < 0.01). The 90- to 179-day stage was lower than the over 179-day stage for progesterone and total progestins (P < 0.025). The values within stages of pregnancy were quite variable, as can be noted from the standard deviations (s) and standard errors (sX) in Table 1.

<table>
<thead>
<tr>
<th>Stage of pregnancy</th>
<th>Total progesterone</th>
<th>Total 20 ϒ-ol</th>
<th>Combination of the progestins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(days)</td>
<td>(No.)</td>
<td>X</td>
</tr>
<tr>
<td>16-89</td>
<td>122</td>
<td>86</td>
<td>16</td>
</tr>
<tr>
<td>90-179</td>
<td>97</td>
<td>82</td>
<td>16</td>
</tr>
<tr>
<td>180 +</td>
<td>92</td>
<td>74</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>74</td>
<td>8</td>
</tr>
</tbody>
</table>

**Correlations.** Unfortunately, from data like these one cannot establish the quantity of hormone being released from the corpus luteum for physiological regulation. A large corpus luteum with a smaller quantity of progestins may release no more progestins per unit time than a much smaller gland with higher levels. Correlations were calculated between corpus luteum weight and quantities of progestins and weights of the ovaries and adrenals (Table 2). The correlations between progestins in the corpus luteum and the weight of ovaries or adrenals were not calculated since these tissues contain, on an average, so little that quantitation is only approximate with the method employed (Table 3).

Table 2 shows that there is a limited relationship between corpus luteum weight and the quantity of progesterone or 20 ϒ-ol per gram of tissue. The correlation of −0.33 and −0.25 between corpus luteum weight and progestins during the last two trimesters of pregnancy may be biologically significant, since this represents about a 2-μg. decrease per 1-g. increase in corpus luteum weight. Quite a different relationship might be observed if enough assays were made on corpora lutea during their growth phases. The data for 16 to 89 days suggest such a possibility.

Cows with larger corpora lutea also tended to have heavier ovaries and heavier adrenals (P < 0.05). This can not be interpreted, since age and size of cow may be some of the determining factors.

Progesterone/gram was not highly correlated (0.21, P < 0.05) with 20 ϒ-ol/gram of corpus luteum. Though approximately 70% of the progestins in a corpus luteum is progesterone, there was a wide and apparently random variation between individual cows at each of the three stages of pregnancy. The biological significance of 20 ϒ-ol is not understood, though it does show biological
<table>
<thead>
<tr>
<th>Comparison</th>
<th>Stage of pregnancy (days)</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16-89</td>
<td>90-179</td>
<td>180+</td>
<td>Common</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.L. weight vs.</td>
<td>(N-2)</td>
<td>(r.)</td>
<td>(N-2)</td>
<td>(r.)</td>
<td>(N-2)</td>
<td>(r.)</td>
<td>(N-2)</td>
<td>(r.)</td>
<td>(N-2)</td>
<td>(r.)</td>
</tr>
<tr>
<td>Progesterone/gram</td>
<td>27</td>
<td>-0.01</td>
<td>31</td>
<td>-0.32</td>
<td>25</td>
<td>-0.21</td>
<td>83</td>
<td>-0.13</td>
<td>87</td>
<td>-0.07</td>
</tr>
<tr>
<td>20 β-ol/gram</td>
<td>27</td>
<td>0.17</td>
<td>31</td>
<td>-0.06</td>
<td>25</td>
<td>-0.17</td>
<td>83</td>
<td>0.02</td>
<td>87</td>
<td>0.09</td>
</tr>
<tr>
<td>Combined progestins/gram</td>
<td>27</td>
<td>0.08</td>
<td>31</td>
<td>-0.33</td>
<td>25</td>
<td>-0.25</td>
<td>83</td>
<td>-0.10</td>
<td>87</td>
<td>-0.01</td>
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<tr>
<td>Ovary weight</td>
<td>14</td>
<td>0.54</td>
<td>16</td>
<td>0.20</td>
<td>17</td>
<td>0.31</td>
<td>47</td>
<td>0.41*</td>
<td>51</td>
<td>0.45*</td>
</tr>
<tr>
<td>Adrenal weight</td>
<td>13</td>
<td>0.30</td>
<td>15</td>
<td>0.33</td>
<td>17</td>
<td>0.29</td>
<td>45</td>
<td>0.30*</td>
<td>49</td>
<td>0.30*</td>
</tr>
<tr>
<td>Ovary weight vs.</td>
<td>(N-2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal weight</td>
<td>13</td>
<td>0.50</td>
<td>15</td>
<td>0.51</td>
<td>17</td>
<td>0.06</td>
<td>45</td>
<td>0.39*</td>
<td>49</td>
<td>0.38*</td>
</tr>
<tr>
<td>Progesterone/gram vs.</td>
<td>(N-2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 β-ol/gram</td>
<td>27</td>
<td>0.18</td>
<td>31</td>
<td>0.08</td>
<td>25</td>
<td>0.12</td>
<td>83</td>
<td>0.14</td>
<td>87</td>
<td>0.21*</td>
</tr>
</tbody>
</table>

* Significant correlation at the 1% level of probability.

* Significant correlation at the 5% level of probability.
TABLE 3
Average quantities of progesterone and 20 β-ol per gram for corpora lutea, ovaries, and adrenals

<table>
<thead>
<tr>
<th>Stage of pregnancy</th>
<th>Weight</th>
<th>Progesterone</th>
<th>( Δ^*-pregnene 20 )β-ol-3-one</th>
<th>Combination of the progestins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(days)</td>
<td>((\text{No.}))</td>
<td>((\text{g.}))</td>
<td>((\text{~g.}))</td>
</tr>
<tr>
<td>Corpora lutea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-89</td>
<td>16-89</td>
<td>29</td>
<td>5.9</td>
<td>2.6</td>
</tr>
<tr>
<td>90-179</td>
<td>90-179</td>
<td>33</td>
<td>5.0</td>
<td>1.3</td>
</tr>
<tr>
<td>180+</td>
<td>180+</td>
<td>27</td>
<td>5.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Total</td>
<td>Total</td>
<td>89</td>
<td>5.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Ovaries</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>16-89</td>
<td>16-89</td>
<td>16</td>
<td>18.2</td>
<td>8.5</td>
</tr>
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<td>4.9</td>
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<td>180+</td>
<td>180+</td>
<td>21</td>
<td>14.4</td>
<td>5.5</td>
</tr>
<tr>
<td>Total</td>
<td>Total</td>
<td>54</td>
<td>15.6</td>
<td>6.5</td>
</tr>
<tr>
<td>Adrenals</td>
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<td></td>
</tr>
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<td>16-89</td>
<td>15</td>
<td>24.7</td>
<td>11.2</td>
</tr>
<tr>
<td>90-179</td>
<td>90-179</td>
<td>18</td>
<td>23.8</td>
<td>7.2</td>
</tr>
<tr>
<td>180+</td>
<td>180+</td>
<td>21</td>
<td>24.2</td>
<td>6.0</td>
</tr>
<tr>
<td>Total</td>
<td>Total</td>
<td>54</td>
<td>24.2</td>
<td>8.0</td>
</tr>
</tbody>
</table>

*Identity not certain for adrenals. In this case the 20 β-ol chromatographed like authentic 20 β-ol but was not further identified.

These averages represent an upper limit, since some samples had less than 3 ~g. in the total sample, which is below the sensitivity of the quantitative assay (31).
activity (38) and is considered a metabolite of progesterone (37). If the latter is entirely true, then the quantities in an individual corpus luteum may be higher or lower, depending on the time between slaughter and rapid freezing of the tissue.

Progestins per gram. Analysis was finally made on progestins per gram of corpora lutea, ovaries, and adrenals, since results from the previous sections do not indicate serious biases in this procedure. The results are shown in Table 3.

The progesterone per gram of corpus luteum averaged 20.4 and this was significantly higher than from 90-179 days (P < 0.01) but not for over 180 days. The 20β-ol averaged 13.2 μg/g, which was also significantly higher than 90-179 or over 180 days of pregnancy (P < 0.005). The 16-89 day group average of 33.6 μg/g of progestins was significantly higher than 90-179 days (P < 0.005), but not distinctly higher than after 180 days of pregnancy (P < 0.25). The corpus luteum after 180 days of pregnancy contained more progesterone (P < 0.10), a similar quantity of 20β-ol, and more total progestins/gram (P < 0.10) than during the interval of 90-179 days of pregnancy.

The mean values listed for progestins of the ovaries and adrenals represent upper limits. Since the limit of quantitative measurement of progestins on a paper chromatogram was about 3 μg., all values falling below 3 μg. were recorded as < 3 μg. and this figure was used for subsequent calculations. Because of the low concentrations of the progestins in ovaries and adrenals, a majority of these samples were in the < 3 μg. group. It is recognized that this treatment will result in a skewed distribution, so that in contrast with the case of corpora lutea, the standard errors for ovaries and adrenals are only approximations.

The combined weight of the ovaries less the weight of the corpus luteum averaged 18.2 g. from 16 to 89 days of pregnancy, which was significantly heavier than ovaries after this time (P < 0.05). The progesterone, 20β-ol, or combined progestins in the ovaries did not vary during pregnancy, nor was there any variation in size or progesterone content of the adrenal glands. Though progestins/gram or adrenal tissue are shown in Table 3, the compound indicated as 20β-ol was not identified beyond its chromatographic properties in the Bush Skellysolve B-95% methanol system. Thus, it may be some other closely related compound.

General discussion. Although progesterone has been isolated and identified as the major progestin in the bovine corpus luteum (11), how essential this structure is to pregnancy maintenance in this species still remains controversial. Attempts to explain the role of the corpus luteum of pregnancy through extirpation have yielded variable results.

Gorski et al. (11) have recently isolated and identified 20β-ol from bovine ovaries. Both the 20α-ol and 20β-ol have been isolated and identified in human ovaries and placentae (24, 29, 36, 37). Since 20β-ol has two times the activity of progesterone in the Hooker Forbes test on mice, and though only one-fifth the activity of progesterone in the Clauberg test on rabbits (37), it may well be of major physiological importance in the bovine. Gorski et al. (12) found that the corpus luteum of pregnancy was the major source of 20β-ol and that
it was present in concentrations of one-fifth to two-fifths that of progesterone. In the present study, progestins in the corpora lutea were 68% progesterone.

Progestosterone has been isolated from extracts of the bovine adrenals by various workers (2, 3, 4, 12) and accordingly has been suggested as an extra-ovarian source of this steroid during pregnancy. In addition, Balfour et al. (3) have reported the isolation and identification of 20 a-ol from the effluent blood of the adrenal gland of young calves. Gorski et al. (12) found relatively low levels of progesterone present in the adrenal as compared with the corpus luteum of pregnancy. The fact that these levels are low (Table 3) does not preclude the notion that the adrenal glands do or may be able to adapt to insufficient supplies of progestins from ovarian sources during later stages of pregnancy. This may explain the inconsistent results observed following removal of the corpus luteum (20) or the entire ovary (7, 26, 27) during late gestation in the bovine.

Although the placenta of various species contain significant quantities of progestins, this does not seem to be true for the bovine (10, 12, 30). However, in one study the placenta of the bovine was implicated as a source of progesterone (22).

The results of our study are similar to those reported to Kaay (17). He found that the progesterone content of the corpus luteum of pregnancy in the bovine was highest during the third month. Contrary to these findings Melampy et al. (22), using a different method, reported that the progesterone content of the corpus luteum and of the residual ovarian tissue were greatest during the period between 90 to 129 days of gestation. He also reported that the progesterone content of the adrenal gland reached a maximum during the period between 170 to 209 days of pregnancy. No similar peak of activity of the adrenal glands was found in this study.

It is apparent from the present data that the corpus luteum as a source of progestins is most critical during the first 3 mo. of pregnancy, and thereafter declines in function. This decline may indicate that: (1) After the third month lower levels of progestins are required by the bovine in maintaining pregnancy to normal birth; (2) a combination of progestins elaborated by the adrenal gland and ovaries is at least partially sufficient for pregnancy maintenance; (3) an extraovarian source of these hormones exists but has as yet not been found or; (4) other progestational compounds exist in the bovine. This study suggests that the corpus luteum remains functional throughout pregnancy and is probably essential throughout for normal maintenance of pregnancy in the bovine, since the levels apparently were increasing during the last trimester of pregnancy.

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REFERENCES

(6) CORNER, G. W., AND ALLEN, W. M. Physiology of the Corpus Luteum. II. Production of a Special Uterine Reaction (Progestational Proliferation) by Extracts of the Corpus Luteum. Am. J. Physiol., 88: 326. 1929.


