HORMONAL AND REPRODUCTIVE PROFILES OF EARLY POSTPARTUM BEEF HEIFERS AFTER PROLACTIN SUPPRESSION OR STEROID-INDUCED LUTEAL FUNCTION

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Summary

Two trials were conducted with 24 primiparous suckled beef heifers to determine independent and combined effects of a prolactin-suppressing agent and exogenous steroids on serum hormones and reproductive activity. Heifers were assigned in groups of six to one of four treatments: 1. (C)-subcutaneous control injections administered once a day for 2 days, followed by 2 days of no injections; sequence repeated from day 5 through day 40 postpartum or until first naturally occurring estrus; 2. (CB)-subcutaneous injections of 80 mg CB-154 (2-bromo-α-ergokryptine) (Sandoz) in ethanol once a day for 2 days, followed by 2 days of no injections; sequence repeated from day 5 through day 40 postpartum or until first naturally occurring estrus; 3. (PE)-intramuscular (IM) injection of 25 mg progesterone in sesame oil on day 15 postpartum, followed by IM injection of 4 mg estradiol-17β in sesame oil 48 hr later, or 4. (CBPE)-treatments 2 (CB) plus 3 (PE). A marked reduction in serum prolactin was observed after a single injection CB-154 in all CB and CBPE treated heifers, reaching minimum concentrations by day 7 postpartum after a second injection on day 6. Continued treatment decreased (P<.01) prolactin concentrations over the 35 day blood sampling period to an average of .9 ± .2 ng/ml as compared to 19.2 ± 5.6 ng/ml for non-CB-154 treated (C and PE) heifers. In non-CB-154 treated heifers, serum prolactin was higher during the spring (33.5 ± .9 ng/ml) than the fall (2.6 ± .9 ng/ml). Treatment CB had no effect on serum concentrations of LH, estradiol-17β or progesterone. The resumption of cyclic activity in C and CB heifers prior to day 40 postpartum was characterized in four of six animals by 4- to 5-day elevations of serum progesterone and 7- to 8-day cycles, which were preceded in every case by peaks of estradiol-17β (9 ± 1.9 pg/ml) and nonstanding estrous behavior (three of four) or no behavior (one of four). Palpable corpora lutea were detected in two of four animals during these progesterone increases. Subnormal luteal function was followed by another estradiol-17β peak (7 ± 2 pg/ml) and estrus (three of four) or nonstanding estrus (one of four). Injection of progesterone followed by injection of estradiol-17β resulted in a preovulatory discharge of LH in 12 of 12 PE and CBPE heifers, with eight of 12 initiating luteal function within 72 hr after the LH surge. On the basis of endocrine data, suppression of endogenous prolactin release did not (P<.05) potentiate steroid treatment effects. Treatments PE and CBPE resulted in a reproductive trend which reflected endocrine findings. Data fail to provide evidence that prolactin is antigonadotropic in bovine heifers.

(Key Words: Postpartum, Heifer, CB-154, Prolactin, Progesterone, Estradiol-17β.)

Introduction

The inhibitory influence of frequent suckling or milking on postpartum ovarian function in the bovine was first described by Clapp


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(1937) and has since been confirmed in both beef (Graves et al., 1968; Oxenreider, 1968; Wiltbank and Cook, 1958) and dairy females (Saiduddin et al., 1968b; Wagner and Oxenreider, 1971). This influence can be lessened but not eliminated by the provision of adequate dietary energy after calving (Wiltbank et al., 1964; Dunn et al., 1969). Short et al. (1972) compared suckled, nonsuckled and nonsuckled-mastectomized cows and concluded that mammary stimulation may prolong the postpartum interval independent of nutrient intake. This concept has recently been supported (Wettetman et al., 1978).

In rats (Amenomori et al., 1970; Ford and Melampy, 1973), women (Rolland et al., 1975b; Villalobos et al., 1976) and monkeys (Maneckjee et al., 1976), high prolactin concentrations in serum during lactation have been correlated with inhibited gonadotropin secretion, suggesting that this polypeptide may play a role in the suckling-induced delay of postpartum ovarian activity. Accordingly, suppression of prolactin release in postpartum women with 2-bromo-α-ergokryptine (CB-154) blocked lactation and caused early restoration of ovulatory cycles (Varga et al., 1972; Rolland et al., 1975a; Villalobos et al., 1976). A similar relationship has been reported in ewes after elimination of the suckling-induced prolactin discharge by mammary denervation (Kann and Martinet, 1975), but a study in the rat is at variance with the interpretation that prolactin mediates this antigonadotropic state (Lu et al., 1976).

In beef cows, hormonal patterns associated with the resumption of normal cyclic activity after calving have been reported (Humphrey et al., 1976) and appear to be similar to those occurring at the onset of puberty (Gonzales-Padilla et al., 1975b). These patterns have been mimicked in prepuberal heifers by single injections of progesterone and estradiol-17β, with the two acting synergistically to promote subsequent ovulation (Gonzales-Padilla et al., 1975a). The following experiment was undertaken to assess the effect of chronic administration of CB-154, sequential injections of progesterone and estradiol-17β on a combination of these treatments on endocrine and reproductive patterns of beef heifers during the early postpartum period.

Materials and Methods

Two trials were conducted with 24 primiparous heifers. The first trial (October-January) involved 12 2-year-olds from the University of Arizona herd, including six Hereford, four Angus and two Hereford × Brown Swiss females. The second trial (January-May) involved 12 3-year-old Herefords previously reared and maintained under range conditions. Average postcalving weights for the two groups were 391 and 395 kg, respectively.

Pre-experimental Period. For 30 to 45 days before calving, animals in each trial were maintained as a group and fed approximately 3.4 kg TDN per head daily. All heifers were considered to be in excellent body condition at calving. During this pre-experimental period, heifers were periodically placed into individual pens (2.4 × 4.9 m), haltered and introduced to the handling and restraint procedures of the study.

Experimental Design and Protocol. Immediately after calving, each heifer and her calf were placed in an individual pen, where both were maintained for the duration of the treatment and bleeding schedule. Cubed alfalfa hay supplying 5.1 kg TDN per head daily was fed to each lactating female during this period. On day 2 postpartum, a permanent indwelling catheter was surgically inserted into the left or right jugular vein and sutured to the skin of the neck. Functional integrity was maintained over the 35-day bleeding schedule by flushing with sterile heparin solution (2,000 μ/ml) after each blood sampling.

Animals in each trial were allotted in a randomized block design to one of four treatments (six heifers per treatment group): 1. (C)-sesame oil and ethanol control injections only; 2. (CB)- 80 mg 2-bromo-α-ergokryptine (CB-154, Sandoz-Wander Inc.) injected subcutaneously in 2 ml of 50% ethanol for 2 consecutive days, followed by 2 days of no injections; sequence repeated from day 5 through 40 postpartum or until first naturally occurring estrus; sesame oil injections also given, on days 15 and 17; 3. (PE)- 25 mg progesterone (Sigma Chemical) injected intramuscularly (IM) in 5 ml sesame oil on day 15 postpartum, followed 48 hr later by injections of 4 mg estradiol-17β (Sigma Chemical) in 2 ml sesame oil; ethanol control injections also given; 4. (CBPE)- treatments CB plus PE as described above. All injections were administered immediately after afternoon blood sampling. Forty milliliters of blood were taken once daily at 1600 hr (trial 1) or 1800 hr (trial 2) on day 5 through 13 (stage 1) and 19 through
40 postpartum (stage III), and every 6 hr on days 14 through 18 postpartum so as to monitor acute changes of LH and prolactin after steroid treatment (stage II). Blood was allowed to clot at 4 C and then centrifuged for separation. Each sample was divided into four aliquots and stored at -20 C until assayed for prolactin, luteinizing hormone (LH), progesterone and estradiol-17β.

Reproductive activity was monitored by removing heifers from their individual pens in the morning and afternoon and placing them with an intact bull of known high fertility for a period of 1 hour. Detailed observations for signs of estrous behavior were conducted, and natural service by the bull was allowed at estrus. No more than six heifers were placed with the bull at one time, and when more than one heifer exhibited signs of estrus, each was separated and given an independent opportunity to be bred.

Examination of the ovaries per rectum was conducted once weekly and used in conjunction with serum progesterone concentration for determination of time of first luteal tissue formation through 40 days postpartum. On day 41, each heifer was returned with her calf to the herd, where an intact bull wearing a chin ball marker was continuously maintained. Twice-daily observations for signs of estrus and breeding activity were continued through 100 days postpartum as were weekly palpations. Intervals to first estrous behavior, standing estrus, ovulation/luteal function and conception were determined throughout this period. Unless otherwise stated, estrous behavior refers to both standing and nonstanding estrus, whereas estrus refers to standing heat only.

Hormone Analyses. Laboratory analyses of prolactin, LH, progesterone and estradiol-17β were performed by radioimmunoassay. Results of all assays were analyzed by a logit-response, log-dose transformation (Rodbard and Lewald, 1970) for estimation of unknown potencies from the standard curve. Unknown estimations in each assay were rejected and reassayed if duplicates deviated from the mean by more than 10%. Weighted least-squares regressions for standard curves and serum dilutions allowed for tests of linearity and parallelism, and appropriate statistics were generated from all assays for determination of specificity, sensitivity, accuracy and precision.

Radioimmunoassay of Estradiol-17β and Progesterone. Serum estradiol-17β was assayed as described by Britt et al. (1974), with the following modification: Serum duplicates of .5, 1.0 or 2.0 ml, depending on expected concentration, were extracted twice with three volumes of redistilled benzene. Recovery estimates were determined in each assay by addition of 3,000 dpm of 3H-2,4,5,6-estradiol (New England Nuclear) to a series of 20 representative samples, followed by incubation at 4 C for 1 hr before solvent extraction. Extraction efficiency averaged 86%. Antiestradiol-17β (Holly Hill Biologicals), raised against the 6-oxime derivative of estradiol-17β-bovine serum albumin, was used at a dilution of 1:10,000. Its specificity has been described previously (Resko et al., 1975) for use in radioimmunoassay of estradiol-17β in monkey plasma. Inhibition curves obtained in our laboratory indicate that this 6-keto system cross-reacts with estrone and estriol to the extent of 3.0 and 1.5%, respectively. Accuracy of the assay as determined by the addition of estradiol-17β to bovine sera yielded a regression coefficient of .99 and a correlation coefficient of .99. Blanks, as determined with triple distilled water and steroid-free serum in each assay, averaged 1.3 ± 1.1 pg and were not subtracted from unknowns. Sensitivity, determined as the lower 95% confidence limit of these blanks, averaged 2.3 ± 1.0 pg, and the intra- and interassay coefficients of variation of two pooled sera in 21 assays were 7.2 and 12.6% respectively.

Progesterone was determined by the procedure described by Louis et al. (1973), with a slight modification of the extraction procedure. Fifty, 100 or 200 µl of serum, depending on expected concentration, were double extracted in duplicate with 2 ml benzene-hexane (1:2) for 3 minutes. For estimation of procedural losses, approximately 3,000 dpm 1,2,6,7-3H-progesterone were added to a representative group (n=20) of tubes in each assay containing the three volumes of bovine sera assayed then incubated for 1 hr at 4 C extracted. Recovery averaged 91%. A rabbit antiserum to progesterone-11-α-bovine serum albumin (Holly Hill Biologicals) was used at a dilution of 1:10,000. Specificity was tested against six closely related steroids with little or no interference observed, and the slopes of curves for extracts of two bovine sera were parallel to that of the standard. Recovery of added amounts of progesterone to cow sera by radioimmunoassay resulted in regression and correlation coefficients of .96 and .99, respectively. Quadruplicate blanks
determined in each assay averaged 10.8 ± 8 pg and were not subtracted from unknowns. The sensitivity of 15 assays averaged 11 ± 4 pg, and the intra- and interassay coefficients of variation were 6.5 and 16.2%, respectively.

Radioimmunoassay of LH and Prolactin. Serum concentrations of LH were measured in samples collected at 6-hr intervals by the double antibody method of Niswender et al. (1969), and highly purified LH (LER-1072-2), iodinated with 125I (New England Nuclear), served as labeled antigen. Samples were assayed in duplicate at 50 or 200 μl per tube, with all final determinations made in one assay after establishment and validation of the system. The first antibody, prepared in rabbits against NIH-LH-B7, was used at a dilution of 1:50,000. The binding characteristics of the antibody and validation for its use in bovine sera have been reported by Golter et al. (1973); results from our laboratory concerning the specificity of this antiserum agree with their report. The second antibody was produced in sheep against rabbit gamma globulin and used in the assay at a dilution of 1:3. Estimates of unknowns were expressed in terms of NIH-LH-B10, and dose-response relationships indicated that the standard curves were parallel to those obtained with three bovine sera. Recovery estimates by radioimmunoassay yielded regression and correlation coefficients of 1.02 ± .01 and .99, respectively. Sensitivity of the final assay from which all values are reported was .06 ng, and the intraassay coefficient of variation was 9.1%.

Prolactin was assayed in serum volumes of 50 to 200 μl in animals not receiving CB-154; volumes as high as 300 μl were tested in animals which received the drug. Radioiodination and radioimmunoassay were performed as described by Davis et al. (1971), with purified ovine prolactin (LER-860-2) used as the labeled antigen. Prolactin antiserum (DJB-7-0330:300), produced in rabbits against NIH-P-S8, was used as first antibody. The use of this antiserum in the radioimmunoassay of ovine prolactin has been described by Echternkamp et al. (1976). The second antibody was the same as that described for the LH assay, and it was used at a dilution of 1:3. Validation experiments for use of the first antibody in bovine sera were performed in this laboratory with three different sera, resulting in inhibition curves parallel to the standard. Except for growth hormone, the system was not influenced by other pituitary hormone preparations. However, as previously reported by Gonzalez-Padilla et al. (1975b) for NIH-GH-B17, the preparation tested in this laboratory (NIH-GH-B18) also exhibited a small degree of cross-reaction; Gonzalez-Padilla et al. (1975b) ascribed this phenomenon to contamination. The assay demonstrated a close agreement between expected and observed recoveries of added mass (b = 1.1 ± .01; r = .99). Precision estimates yielded an intraassay coefficient of variation of 7.6% and an interassay coefficient of variation of 10.3%. The sensitivity of five assays averaged .13 ± .08 nanograms.

Statistical Analyses. Hormonal data, transformed to log10 to minimize heterogeneity of variance, were analyzed by split-plot analyses (Gill and Hafs, 1971). Comparison of means among treatments were made by Duncan's new multiple range test, while within-treatment comparisons were made with paired t-tests (Steel and Torrie, 1960).

Results

Ovulation, Estrus and Endocrine Response. Serum prolactin, progesterone and estradiol-17β did not differ (P>.05) among experiment groups on day 5 postpartum prior to initiation of treatment in either trial. Pooled mean levels ± SE were 14.3 ± 3.9 ng/ml, .09 ± .01 ng/ml and 3.1 ± .7 pg/ml, respectively. A precipitous decline in serum prolactin was noted after a single injection of CB-154 in all CB and CBPE heifers, reaching minimum levels by day 7 postpartum after a second injection on day 6 (figures 1 through 3). Continued treatment decreased (P<.01) prolactin in these groups, with mean values of .8 ± .1 and .9 ± .2 ng/ml for groups CB and CBPE vs 17.2 ± 3 and 21.1 ± 6.4 ng/ml for groups C and PE, respectively (table 1). In accordance with experimental design, CB-154 administration was discontinued (days 33 or 34) in three CBPE-treated heifers which exhibited standing estrus at the end of steroid-induced first cycles. However, prolactin in these heifers remained suppressed throughout the blood sampling period. A comparison of serum prolactin in non-CB-154-treated groups by trial revealed higher (P<.05) levels in heifers during the spring trial than during the fall trial. Mean ± SE prolactin during the fall was 2.6 ± .9 ng/ml vs 33.5 ± 9.1 ng/ml during the spring.

Mean serum hormone concentrations over all stages of the experiment are presented in table 1. The information is summarized by treatment
Treatment CB had no effect (P > .05) on circulating levels of progesterone and estradiol-17β when compared to control treatment C during any stage of the experiment, nor on levels of LH measured during stage II. The hormonal patterns associated with the resumption of cyclic activity in these groups are shown in figure 1. Segment I represents profiles by treatment for CB (n=2) and C (n=3) during the first 3 weeks postpartum. Segment II shows data normalized to the day of the first cycle and averaged over treatments for estradiol-17β and progesterone, with prolactin presented for each treatment. Since cyclic activity did not resume in one CB-treated animal until day 38 postpartum, she was not included in segment II. Hormone concentrations during segment I in this group of animals appeared similar to those animals which did not resume cyclic activity (figure 2). However, inspection of the data (table 1) shows that circulating estradiol-17β was consistently higher (P < .01) in animals which began to exhibit luteal function (LF) during the next 19 days. Although not tabulated, this trend (P < .01 to P < .05) was also evident at each stage (I and II) before initiation of cyclic activity (stage III). It should be noted that the significant differences in mean hormone concentrations between LF and NLF categories of treatments C and CB are completely confounded with trial. All heifers in these two groups which exhibited LF within 40 days belonged to trial I, during which prolactin was lowest in C heifers and basal estradiol-17β was highest in both C and CB heifers.

The information in segment II of figure 1 shows a 4- to 5-day increase of progesterone, the mean peak value of which did not exceed .7 ng/ml during an initial 7- to 8-day cycle. These brief increases were perceded in every case by a serum estradiol-17β peak averaging ± SE 9.0 ± 1.9 pg/ml, with either nonstanding estrous behavior (three or four) or no behavior (one of four) observed. Weekly palpation allowed for the detection of CL in two of the heifers showing the abbreviated progesterone increases, but palpation did not coincide with these
Figure 2. Hormonal profiles of three CB-154 (CB) and three control (C) heifers which failed to exhibit estrus or luteal function through 40 days postpartum. – Arrows indicate CB-154 or control injection.

Increases in the other two; therefore, ovarian morphology was not ascertained. A second estradiol-17β peak occurred at the end of this first cycle in all animals represented in figure 1, segment II. The mean value ± SE observed for these peaks was 7.0 ± 2 pg/ml and was followed the next day (day 0) by standing (three of four) or nonstanding estrus (one of four). The C heifer which exhibited nonstanding estrus at this time showed a second 5-day progesterone increase and a 9-day cycle before standing heat occurred. The heifer excluded from segment II of figure 1 because of incomplete endocrine data exhibited increasing serum progesterone on day 40 and a return to estrus after an 8-day cycle, suggesting that the endocrine events occurring in this animal were similar to those depicted for the others. However, one CB-treated heifer which is not shown in figure 1 or 2 showed estrous behavior on day 28 postpartum, followed by a 14-day cycle and standing heat on day 42. Serum progesterone was 4.6 ng/ml at midcycle, and a CL was detected by palpation. No endocrine or behavioral events indicative of cyclic activity were noted before day 28 in this individual.

Profiles associated with steroid-induced luteal function in treatments PE and CBPE are shown in figure 3. Segment I depicts profiles for all animals by treatment through 21 days postpartum. Segment II represents concentrations during induced first cycles for eight of 12 heifers responding to treatment. Progesterone and estradiol-17β remained low in both PE and CBPE animals through day 13 postpartum in single daily bleedings (stage I). Mean estradiol-17β for this period was 2.7 (PE) and 3.4 pg/ml (CBPE), respectively. Progesterone averaged 0.08 and 0.06 ng/ml, respectively, with prolactin concentrations high and variable in PE (12.6 ng/ml) compared to CBPE animals (3.2 ng/ml). Mean prolactin for stage I includes the high concentrations occurring on day 5 postpartum prior to CB-154 treatment.

IM injection of 25 mg progesterone on day 15 postpartum resulted in an increase in serum progesterone within 6 hr, which persisted above control concentrations (P<.001) for an average of 66 hr (figure 3). Highest peaks occurred at 6 or 12 hr postinjection and averaged 595 ± 47 pg/ml, although 6-hr bleedings probably do not reflect highest peak values actually attained. Serum LH and estradiol-17β concentrations did not deviate from C concentrations (P>.05) before or after progesterone treatment. Prolactin concentration fluctuated episodically both before and after progesterone injection among PE animals but was higher (P<.05) than
C concentrations only at 18 hr after progesterone injection and did not exceed (P > .05) the pretreatment PE mean. Prolactin in treatment CBPE remained at or near 1 ng/ml.

Injection of estradiol-17β 48 hr after progesterone priming (day 17) resulted in an increase in serum estradiol-17β at 6 hr post treatment (figure 3). The mean peak concentration ± SE measured was 275 ± 32 pg/ml, with increases persisting above (P < .001) C and pretreatment PE and CBPE concentrations for approximately 30 hours. Administration of estradiol-17β initiated and maintained a substantial (P < .01 to P < .05) increase in serum prolactin at 6, 12 and 18 hr after injection in treatment PE but failed to elicit a measureable change from baseline in prolactin concentrations of CBPE-treated animals (figure 3). An LH surge ranging from 7.7 to 92.5 ng/ml was detected in all PE and CBPE animals at 24 or 30 hr postestradiol, with LH in C and CB-treated heifers remaining at baseline. Luteal function as assessed by increasing serum progesterone was apparent within 72 hr after the LH peak in eight of 12 animals, with highest serum progesterone during induced first cycles averaging 1.7 ± .4 ng/ml. Duration of luteal function in all but one case was abbreviated (12 ± 1.1 days). Thus, six of the eight induced cycles were 14 to 16 days in length; the CL in one heifer regressed even more rapidly, resulting in a 10-day cycle. The estradiol-17β peak at the return estrus of responsive heifers averaged ± SE 9.2 ± 2 pg/ml and was accompanied on the following day (day 0) by standing heat in all cases. All second cycles were normal, averaging 17 to 21 days. The temporal responses of steroid-treated animals are summarized in table 2, including

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**Figure 3.** Hormonal profiles of heifers receiving progesterone and estradiol-17β (PE) or CB-154 plus PE (CBPE). — Segment I depicts profiles for PE (n=6) and CBPE (n=6) through day 21 postpartum. Segment II shows normalized data points for PE (n=3) and CBPE (n=5) in which cyclic activity was induced.
**TABLE 1. MEAN CONCENTRATIONS OF ESTRADIOL-17\(\beta\), PROGESTERONE AND PROLACTIN BY TREATMENT AND CATEGORY OVER ALL STAGES (DAY 5 TO 40) AND OF LH DURING STAGE II (DAY 14 TO 18)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Categ.a</th>
<th>No. anim.</th>
<th>17(\beta)-E(_2) (pg/ml)</th>
<th>Prog. (ng/ml)</th>
<th>Prol. (ng/ml)</th>
<th>LH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>LF</td>
<td>3</td>
<td>3.3(\text{c}) .(\text{f})</td>
<td>.11</td>
<td>4.0(\text{d})</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>NLF</td>
<td>3</td>
<td>1.5(\text{d})</td>
<td>.11</td>
<td>30.3(\text{e})</td>
<td>2.5</td>
</tr>
<tr>
<td>CB</td>
<td>LF</td>
<td>3</td>
<td>3.3(\text{c}) .(\text{f})</td>
<td>.22(\text{c})</td>
<td>.9</td>
<td>2.9</td>
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<tr>
<td></td>
<td>NLF</td>
<td>3</td>
<td>2.7(\text{f})</td>
<td>.14</td>
<td>.8(\text{f})</td>
<td>2.6</td>
</tr>
<tr>
<td>PE</td>
<td>LF</td>
<td>3</td>
<td>10.6(\text{c})</td>
<td>.44(\text{c})</td>
<td>30.1(\text{e})</td>
<td>5.1(\text{c})</td>
</tr>
<tr>
<td></td>
<td>NLF</td>
<td>3</td>
<td>12.2(\text{b})</td>
<td>.25</td>
<td>12.0(\text{d})</td>
<td>2.4(\text{b})</td>
</tr>
<tr>
<td>CBPE</td>
<td>LF</td>
<td>5</td>
<td>19.7(\text{b})</td>
<td>.31</td>
<td>.8(\text{f})</td>
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</tr>
<tr>
<td></td>
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<td>1</td>
<td>12.3(\text{a})</td>
<td>.28</td>
<td>1.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

\(a\) LF (luteal function within 40 days); NLF (no luteal function).
\(b, c(p<.05)\) in columns within treatment.
\(d, e(p<.01)\) in columns within treatment.
\(f, g(p<.01)\) in columns between treatments.

LH response, behavior, luteal function and cyclic activity.

**Postpartum Intervals.** Table 3 gives the averages and standard deviations of postpartum intervals. Because of limited numbers, statistical comparisons were not performed on these parameters. At the end of the experiment, four of six CB and four of six C heifers had begun cyclic activity, with two of the CB and four of the controls conceiving. Mean intervals to conception for these two groups were 61 and 64 days, respectively. However, all PE and CBPE heifers had resumed estrous cycles by day 60, with eight of the 12 having begun on day 18 or 19.

**TABLE 2. TEMPORAL RESPONSES OF POSTPARTUM HEIFERS TO INJECTIONS OF PROGESTERONE AND ESTRADIOL-17\(\beta\) IN TREATMENTS PE AND CBPE**

<table>
<thead>
<tr>
<th>Item</th>
<th>PE</th>
<th>CBPE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>6</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>LH release:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak 24 hr post-E(_2), No.</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Peak 30 hr post-E(_2), No.</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Mean ± SE highest peaks, ng/ml</td>
<td>35.3 ± 13.4</td>
<td>20.7 ± 3.4</td>
<td>27.7 ± 6.7</td>
</tr>
<tr>
<td>Behavior 12 to 36 hr post-E(_2):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperactive only, no.</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Estrus and bred, no.</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Luteal function post-E(_2):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detected by palpation, no.</td>
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<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Detected by serum prog., no.</td>
<td>3</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Mean highest ± SE prog., ng/ml</td>
<td>1.4 ± .1</td>
<td>1.9 ± 1.1</td>
<td>1.7 ± .4</td>
</tr>
<tr>
<td>Mean length ± SE prog. elevation, days</td>
<td>11.3 ± .7</td>
<td>12.4 ± 2.0</td>
<td>12.0 ± 1.1</td>
</tr>
<tr>
<td>Cyclic activity:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Return estrus, no.</td>
<td>3</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Mean length ± SE first cycle, days</td>
<td>14.7 ± .7</td>
<td>16.4 ± 3.0</td>
<td>15.8 ± 1.9</td>
</tr>
<tr>
<td>Treatment</td>
<td>(No)</td>
<td>EB Avg</td>
<td>SD</td>
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</tr>
<tr>
<td>C</td>
<td>(3)</td>
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<tr>
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</tr>
<tr>
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<td>17.7</td>
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</tr>
<tr>
<td>CBPE</td>
<td>(3)</td>
<td>17.7</td>
<td>6</td>
</tr>
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<td>C</td>
<td>(3)</td>
<td>30.0</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>CBPE</td>
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</tr>
<tr>
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<tr>
<td>CBPE</td>
<td>(6)</td>
<td>17.8</td>
<td>0.4</td>
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*One heifer developed metritis.
19 postpartum. Five of six PE and six of six CBPE heifers conceived within 100 days postpartum, with mean intervals to conception of 56 and 54 days, respectively. One PE heifer which began cyclic activity on day 18 failed to conceive because of chronic endometritis and had cycled four times by day 100. Although two more apparent ovulations occurred in treatment CBPE than in PE, the endocrine profiles before, during and immediately after steroid injections were practically identical, including estradiol-induced LH release. Furthermore, luteal phase progesterone concentrations did not differ (P>.05) between responsive PE and CBPE animals. However, among PE-treated heifers, overall mean concentrations of LH during stage II were higher (P<.05) in responsive than in nonresponsive heifers (table 1).

**Discussion**

The dramatic reduction in serum prolactin observed in this study after CB-154 treatment supports previous findings (Karg *et al.*, 1972; Schams *et al.*, 1972; Smith *et al.*, 1974) regarding the potent prolactin-inhibiting activity of this compound in cattle. Also in agreement is the finding that prolactin remained suppressed for at least 6 days after discontinuation of treatment.

Although specific measures of milk production and calf performance were not conducted, all CB-154-treated heifers in the present study continued to lactate, and differences in calf growth among treatments were not apparent. These observations tend to support the contention (Karg and Schams, 1974) that prolactin is primarily lactogenic in the bovine. However, the possible galactopoietic effects of very low circulating levels of prolactin cannot be dismissed.

The difference (P<.05) in serum prolactin noted between non-CB-154-treated heifers in trial I (October-January) and trial II (January-April) may be accounted for by several possible factors. Previous workers have demonstrated an increase in serum prolactin of dairy cattle during spring and summer (Schams, 1972; Karg and Schams, 1974; Koprowski and Tucker, 1973) and in response to increasing ambient temperature (Tucker and Wetterman, 1976). More recently, Peters and Tucker (1978) have shown that increasing photoperiod promotes prolactin secretion in dairy heifers at temperatures above 0°C, with photoperiod and temperature exhibiting a marked synergistic effect at temperatures above 21°C. Consonant with these observations, trial 2 of our experiment was conducted under the influence of increasing photoperiod and ambient temperature, the latter of which is marked under desert Southwest conditions. However, collection stress (Karg and Schams, 1974) cannot be discounted as a possible source of variation since animals in trial 2 were derived from a herd of range cattle which had not been handled as extensively as the cattle used in trial 1. The design of the current experiment does not lend itself to a resolution of this question.

The estradiol-induced prolactin release observed in non-CB-154-treated heifers agrees with the findings of Karg and Schams (1974). However, in the latter study, prolactin release occurred upon removal of the estrogen stimulus after continuous infusion. No major changes occurred in serum prolactin in response to 25 mg progesterone, except at 18 hr postinjection. This may have been a result of a negative dose-dependent pattern previously reported (Karg and Schams, 1974) in which 10 mg, but not 40 mg, of progesterone caused a dramatic increase in serum prolactin. Also, the frequency of blood sampling may not have been adequate to detect acute, short-duration changes. On the basis of 6-hr samples, neither estradiol-17β nor progesterone had a measurable effect on serum prolactin in treatment CBPE. These results are similar to those obtained by Schams (1972), who found that the effects of thyrotropin releasing hormone, a potent prolactin-releasing agent, was essentially blocked as a result of CB-154 treatment.

Although one-half of the CB-treated heifers (three of six) in our trials resumed cyclic activity within 40 days postpartum, inhibition of prolactin release failed to enhance ovarian activity over that of C heifers or to alter the levels of estradiol-17β, progesterone and LH. The results therefore fail to support the hypothesis that prolactin is antigonadotropic in the bovine. This finding is in contrast to the those obtained in studies of women (del Poza *et al.*, 1972; Varga *et al.*, 1972; Rolland *et al.*, 1975a), in which suppression of endogenous prolactin release with CB-154 restored ovarian function early in the postpartum period, and in studies of rats (Amenomori *et al.*, 1970; Chen and Meites, 1970), monkeys (Maneckgee *et al.*, 1978) and women (Villalobos *et al.*, 1976), which have correlated high levels of prolactin during lactation with inhibited gonadotropin
secretion. In these species, prolactin is both lactogenic and galactopoietic. Thus, the relative contributions of prolactin, suckling and lactation toward ovarian inactivity are difficult to separate. However, in a study in which the suckling stimulus was maintained on CB-154-treated postpartum rats (Lu et al., 1976), strong evidence was obtained to suggest that the mechanism by which suckling inhibits gonadotropin secretion is completely separate from that which stimulates prolactin release.

To our knowledge, the estradiol-17β peaks reported herein which were found to precede abbreviated increases in serum progesterone during the first 3 to 4 weeks postpartum have not been previously reported, but are similar to those observed at normal estrus (Wetteman et al., 1972). However, the occurrence of 3-to 5-day increases of circulating progesterone before the resumption of normal length cycles has been reported in both postpartum dairy (Pope et al., 1969; Robertson, 1972; Erb et al., 1971) and beef cows (Arie et al., 1974; Lavoie and Moody, 1976; Humphrey et al., 1976). A similar profile has been shown to exist at the attainment of puberty in the beef heifer (Gonzalez-Padilla et al., 1975b), and these fluctuations were associated with a so-called “priming” and “prepuberal” LH peak. The results of these studies imply that the establishment/reestablishment of normal cyclic activity is a gradual process involving a functional integration of the hypophyseal-hypothalamic-ovarian axis and appears to be common to both prepuberal and postpartum females. However, the results of the studies failed to provide sufficient evidence either to confirm or to disprove that ovarian cyclic activity and luteal tissue formation are the origin of short-term progesterone increases. It has been speculated by some that at least the initial increases in the prepuberal heifer may be of adrenal origin (Gonzalez-Padilla et al., 1975b). The report by Castenson et al. (1976) does not support this concept; rather, it provides clear evidence that the initial increases, at least in the postpartum heifer, are the result of luteal tissue formation and, in most cases, of ovulation. This evidence is supported by the information from our study, including the detection of CL (two of four) during 4-to 5-day progesterone elevations and initial 7-to 8-day cycles preceded and followed by estradiol-17β peaks, no estrous behavior, nonstanding estrous behavior or estrus. It should be noted that one nonsteroid-treated heifer in the current study exhibited a 14-day first cycle instead of a 7- to 8-day cycle. This suggests that not all first cycles are necessarily of such short duration. Furthermore, steroid-induced luteal function in treatments PE and CBPE resulted in first cycle lengths which in most cases approached normality (X = 15.8 days). This observation suggests that endogenous concentrations of ovarian steroids are extremely important in the modulation of subsequent luteal function.

A single injection of 4 mg estradiol-17β given 48 hr after presensitization with progesterone resulted in a preovulatory discharge of LH similar to that seen on the day of estrus in cows and heifers (Christenson et al., 1974). The discharge led to the establishment of luteal function in eight of 12 treated animals. The pattern of response is practically identical to that observed in prepuberal heifers given 2 mg estradiol-17β after progesterone (Gonzalez-Padilla et al., 1975a), except that the LH peak occurred 6 to 12 hr later in our study. This variation in temporal response may be dose-related, but the relationship apparently is not linear, since a 10-mg dose of estradiol-17β resulted in an LH surge 23 hr after injection in spayed cows (Short et al., 1973), a time relationship similar to the one we observed after injection of 4 milligrams. Estrogen-induced LH release is diminished during the luteal phase of cows (Hobson and Hansel, 1972), and pituitary response to GnRH is also decreased during midcycle in ewes (Reeves et al., 1971). However, exogenous progesterone fails to inhibit LH release when exogenous estradiol is administered concomitantly (Short et al., 1973; Swanson, 1974; Hausler and Malven, 1976), and presensitization with exogenous progesterone appears to mimic normally-occurring changes and to enhance ovulation induction with estrogen in rats (Docke and Dörner, 1966; Ying and Greep, 1971; Caligaris et al., 1972) and heifers (Gonzalez-Padilla et al., 1975a). The mechanism by which progesterone acts synergistically with estradiol to promote ovulation remains unknown, but evidence (Gonzalez-Padilla et al., 1975a,b) indicates that the ovary may be the site of action since pituitary LH releases of similar magnitude can be caused by estradiol with or without progesterone pretreatment.

The current study suggests that the pituitary of the postpartum beef heifer is competent by at least 2 to 3 weeks postpartum to release.
preovulatory surges of LH. These findings are therefore similar to those confirmed in dairy cows (Britt et al., 1974; Kesler et al., 1977; Fernandes et al., 1978). In view of these results, as well as those of Saiduddin et al. (1968a) in postpartum beef cows and of Thatcher and Wilcox (1973) in dairy cows, it appears that procedures designed to enhance rebreeding efficiency can and should begin within 2 to 3 weeks postpartum. The beneficial effects of steroid-induced luteal function in our study supports this concept, but endocrine data and reproductive trends fail to provide significant evidence that suppression of endogenous prolactin release can potentiate those effects.

Literature Cited


