Modulation of reproductive hormones by suckling and exogenous gonadal hormones in young beef cows

post partum

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Summary. Plasma concentrations of LH, prolactin, progesterone and oestradiol-17β were measured during post-partum anoestru in 12 young beef cows (6 suckling, 6 not suckling). A progesterone-releasing intravaginal device (PRID) was inserted on Day 60 post partum for 12 days in 3 cows in each group. Two of three suckling cows exhibited oestrus within 2 days after PRID removal (Day 73) whereas none of the untreated cows showed oestrus before Day 93. Five of six non-suckling cows showed oestrus before Day 60.

Intensive bleeding periods on Days 45, 58, 70 and 72 provided plasma samples for LH and prolactin measurements. Suckling did not affect the LH baseline or the number of LH spikes but did decrease the mean value of the spikes; all three of these characteristics were increased for prolactin. The number of prolactin spikes and the length of anoestru in suckling cows were correlated \( r = +0.87, P < 0.05 \) as were mean basal LH level and length of anoestru \( r = -0.89, P < 0.05 \). A decrease in plasma LH occurred in suckling and non-suckling cows during the period (Days 60–72) they were treated with gonadal hormones.

Introduction

Return to oestrus in suckling cows is delayed compared with that of cows milked 2 or 4 times daily (Clapp, 1937; Wiltbank & Cook, 1958). Early weaning of calves on Days 10 or 30 post partum shortens the post-partum anoestru by 20 (Bellows, Short, Urick & Pahnish, 1974) or 8 days (Smith & Vincent, 1972), respectively. Suckling causes hyperprolactinaemia in many species. Schams (1972) has shown that milking, oxytocin and teat stimulation elevate plasma prolactin levels in the cow. Less is known of the effect of suckling on the plasma LH levels in the cow. Carter, Dierschke, Rutledge & Hauser (1980) bled multiparous beef cows once daily for 25 days post partum and LH concentrations were lower in suckling than in non-suckling cows and there was an average of 0 and 2 ovolutions per cow, respectively. GnRH-induced LH-response curves were greater for non-suckling than for suckling cows. When machine milking of dairy cows was continued, suckling cows had lower mean serum LH concentrations on Day 13 post partum than did non-suckling cows; this was due to a reduced frequency and lower amplitude of episodic LH peaks (Carruthers, Convey, Kesner, Hafs & Cheng, 1980).

Exogenous gonadal hormones can shorten the post-partum anoestrous period; a decrease occurred with oestradiol injections alone or progesterone treatment (Ulberg & Lindley, 1960). Oestrogen and progesterone given a few days after calving shorten the anoestrous period (Oxenreider, 1968). Little is known of the effects of oestradiol-17β and progesterone treatment during the post-partum period on LH and prolactin secretion.
In this study beef cows were bled intensively at intervals during post-partum anoestrus to examine the effects of suckling and progesterone implants on LH and prolactin secretion.

**Materials and Methods**

**Treatments**

Twelve (12) cross-bred beef cows, 2 or 3 years of age, that had calved between 1 January and 15 March were used. They were healthy but not in full body condition. The experiment began on Day 32 after parturition (= Day 1) with 6 cows being assigned to Group NS (not suckling) because the calves were removed at birth. Group S (suckling) contained 6 cows, each with her own calf. Careful observation showed that there were no calves that had developed the habit of suckling other cows. The cows were observed continuously and all suckling episodes were recorded. All cows were fed an adequate diet of grain and coastal bermuda grass. They were observed for oestrus daily from 07:00 to 07:30 h and from 16:00 to 16:30 h. A cow was considered to be in oestrus when she stood for mounting by another cow. All cows were observed for oestrus between Days 32 and 95, except for Cow 2, which was observed between Days 46 and 95.

Half the cows in each group were treated with a progesterone-releasing intravaginal device (PRID: Abbott Laboratories, North Chicago, Illinois) which contained 6·75% progesterone by weight and had a surface area of 76·2 cm². The other cows in each group received a device containing no progesterone (dummy). The PRID was inserted on Day 60 after parturition and removed on the morning of Day 72. Cows which had received a PRID were given an i.m. injection of 6 mg oestradiol-17ß valerate in saline on Day 60 and were inseminated 56 h after removal of the device. Cows receiving the dummy device were inseminated 12 h after the first detected oestrus following Day 72.

All cows were bled via a jugular vein catheter on Days 45, 58, and 70 at 30-min intervals for 24 h and on Days 72–73 for 36 h at 30-min intervals. Once daily throughout the experiment a blood sample was taken from each cow for assay of gonadal hormones. Plasma was stored at −20°C until analysis.

**Hormone assays**

LH was measured by a specific double-antibody RIA using an anti-bovine LH serum (GDN-B225) as described by Henricks, Dickey & Niswender (1970). Triplicate standard curves were run with NIH-LH-B5 in each assay, and results were expressed in terms of ng NIH-LH-B5. Highly purified bovine LH (LER-1072-2) was used for iodination. In this system 36·7 ± 2·5 (s.e.m.)% (n = 12) of the radioiodinated LH was bound to the antibody in the absence of unlabelled hormone. All plasma samples were run in duplicate at 200 μl per assay tube. The minimum detectable quantity was 0·09 ± 0·02 ng LH per assay tube. Interassay coefficients of variation (CV) of 14·3% (n = 12) and 11·3% (n = 12) were obtained for two separate pools of plasma taken on the day of oestrus.

Prolactin concentration was determined by a double-antibody system similar to that for the LH assay, but using a specific anti-bovine prolactin serum (Schams, 1972). Triplicate standard curves were run with NIH-P-B4 in each assay, and values were expressed in terms of ng NIH-P-B4. Highly purified ovine prolactin (LER-860-2) was used for iodination. In this system 50 ± 2% (n = 12) of the radioiodinated ovine prolactin was bound to the antibody in the absence of unlabelled hormone. The sample volume was 20 μl and duplicate measurements were made of all samples. The sensitivity limit of the assay was 0·34 ± 0·20 ng (n = 12) per assay tube. The CVs for four plasma pools were 16·0 (n = 11), 19·9 (n = 12), 18·4 (n = 12) and 17·7% (n = 2) for samples taken at 1, 10, 30 and 60 min after suckling, respectively.
Progesterone was measured in liquid-phase RIA by the method described by Rawlings, Kennedy, Chang, Hill & Henricks (1977), except that a second antiserum was used to separate bound from free hormone. The recovery of progesterone added to plasma was 82 ± 3% (n = 10). Aliquots (200 μl) of plasma were run in duplicate in each assay. The interassay CVs for two pools were 16.5 (n = 15) and 26.3% (n = 15). The sensitivity limit of the assay was 9.1 ± 3.1 pg (n = 12) per assay tube.

Oestradiol-17β was measured according to the method reported by Henricks, Rawlings & Ellicott (1977), except that a second antibody was used to separate bound from free hormone. The recovery of oestradiol-17β added to plasma was 95 ± 1% (n = 12). Extracts were assayed in duplicate and were corrected for procedural losses. The interassay CV for one pool of plasma was 4.0% (n = 12). The sensitivity limit of the assay was 4.7 ± 1.3 pg (n = 11) per assay tube.

Statistical analysis

Significant elevation of LH and prolactin was determined as follows. Any value greater than the mean plus two standard deviations was identified and these figures were deleted from the mean value to give a corrected mean. Values greater than the corrected mean plus two standard deviations were then designated as spikes. The baseline was calculated as the average level after all spike values had been deleted. The baseline, mean spike height and number of spikes were calculated for each cow per bleeding period. These data were analysed in a completely randomized split-plot analysis of variance with a factorial arrangement between treatment (suckling versus no suckling) and implant (PRID versus dummy device). Bleedings were fitted as subunits and partitioned into non-orthogonal polynomial regression.

Results

A summary of the occurrence of oestrus and pregnancy for all the cows is given in Table 1.

Table 1. Incidence of oestrus and pregnancy in suckling (S) and non-suckling (NS) young beef cows treated with a progesterone-releasing intravaginal device (PRID) and oestradiol valerate (E2V) after parturition (Day 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cow no.</th>
<th>Sub-treatment</th>
<th>Day of oestrus before sub-treatment</th>
<th>Day of oestrus after sub-treatment</th>
<th>Day of A.I.</th>
<th>Day pregnancy confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>2</td>
<td>PRID + E2V</td>
<td>—</td>
<td>73</td>
<td>74</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>PRID + E2V</td>
<td>—</td>
<td>73</td>
<td>74</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>PRID + E2V</td>
<td>—</td>
<td>74</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Dummy PRID</td>
<td>—</td>
<td>93</td>
<td>94</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Dummy PRID</td>
<td>—</td>
<td>93</td>
<td>94</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Dummy PRID</td>
<td>—</td>
<td>93</td>
<td>94</td>
<td>139</td>
</tr>
<tr>
<td>NS</td>
<td>7</td>
<td>PRID + E2V</td>
<td>59</td>
<td>73</td>
<td>74</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>PRID + E2V</td>
<td>55</td>
<td>74</td>
<td>74</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>PRID + E2V</td>
<td>60</td>
<td>74, 82</td>
<td>74</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Dummy PRID</td>
<td>38, 57</td>
<td>75</td>
<td>75</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Dummy PRID</td>
<td>—</td>
<td>89</td>
<td>89</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Dummy PRID</td>
<td>34, 55</td>
<td>79</td>
<td>79</td>
<td>124</td>
</tr>
</tbody>
</table>

Gonadal hormones

Progesterone concentrations were < 1 ng/ml for Group S cows until Day 60 (Text fig. 1a). During the PRID treatment of Cows 2, 3 and 5, progesterone rose to 12, 9 and 4 ng/ml.
respectively, whereas in Cows 1, 4 and 6 concentrations remained < 1 ng/ml. Cows 2 and 3 also exhibited oestrus, were inseminated and became pregnant. Progesterone concentrations were lower in Cow 5 during treatment and this cow did not show oestrus or form a corpus luteum.

**Text-fig. 1.** Plasma oestradiol-17β and progesterone concentrations in (a) suckling and (b) non-suckling cows from Days 45 to 95 post partum. Between Days 60 and 72 Cows 2, 3, 5, 7, 10 and 11 were treated with a PRID and Cows 1, 4, 6, 8, 9 and 12 were fitted with a dummy device. E = oestrus.
Judging by the plasma progesterone levels, Cows 8 and 12 had an oestrous cycle; they each exhibited oestrus twice before Day 60 and had luteal-phase levels of progesterone between Days 60 and 72. Plasma progesterone was <1 ng/ml in Cows 9, 7 and 11 before Day 60. Cow 10 had increased progesterone values and showed oestrus on Day 55. During PRID treatment plasma progesterone in this cow rose to very high levels (16 ng/ml), probably the sum of the cyclic and implant-released progesterone. In Cows 7, 10 and 11 oestrus occurred within 1–2 days after implant removal.

Plasma oestradiol-17β concentrations in Group S cows were similar to those in Group NS. The sporadic peaking above basal levels of 6–8 ng/ml suggested a periodic growth of follicles. In both groups the peak levels varied between 12 and 16 ng/ml before Day 60. The concentrations rose in response to the injection of oestradiol valerate in all cows and the peaks were greater than those before PRID treatment. Analysis of the hourly level of oestradiol during the 36-h period after implant removal showed that in the treated cows of Groups S and NS oestradiol was higher (P < 0-01) than in the untreated cows; in 5 of the 6 treated cows an oestradiol surge coincided with oestrus.

Pituitary hormones

The plasma concentrations of LH and prolactin for one cow from each group are depicted in Text-fig. 2. Mean numbers of spikes per period and mean basal spike concentrations for LH and prolactin for Groups S and NS are summarized in Table 2.

Table 2. Mean baseline, mean number of spikes and mean spike height (per bleeding period) of LH and prolactin in suckling (S) and non-suckling (NS) cows

<table>
<thead>
<tr>
<th>Group</th>
<th>Cow no.</th>
<th>LH P1</th>
<th>LH P2</th>
<th>LH P3</th>
<th>LH P4</th>
<th>LH Mean</th>
<th>Prolactin P1</th>
<th>Prolactin P2</th>
<th>Prolactin P3</th>
<th>Prolactin P4</th>
<th>Prolactin Mean</th>
</tr>
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<tbody>
<tr>
<td>Mean baseline (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>6</td>
<td>1.1</td>
<td>1.1</td>
<td>0.8</td>
<td>1.0</td>
<td>1.0</td>
<td>55</td>
<td>74</td>
<td>100</td>
<td>110</td>
<td>85</td>
</tr>
<tr>
<td>NS</td>
<td>6</td>
<td>1.1</td>
<td>1.4</td>
<td>1.1</td>
<td>1.7</td>
<td>1.3</td>
<td>42</td>
<td>33</td>
<td>55</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>Mean no. of spikes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>S</td>
<td>6</td>
<td>5.0</td>
<td>4.2</td>
<td>3.8</td>
<td>4.7</td>
<td>4.4</td>
<td>18</td>
<td>16</td>
<td>22</td>
<td>21</td>
<td>19</td>
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<tr>
<td>NS</td>
<td>6</td>
<td>4.1</td>
<td>5.2</td>
<td>4.0</td>
<td>3.0</td>
<td>4.1</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Mean peak height (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>6</td>
<td>2.9</td>
<td>2.1</td>
<td>2.0</td>
<td>1.8</td>
<td>2.2</td>
<td>239</td>
<td>276</td>
<td>279</td>
<td>333</td>
<td>282</td>
</tr>
<tr>
<td>NS</td>
<td>6</td>
<td>3.3</td>
<td>4.0</td>
<td>2.4</td>
<td>5.0</td>
<td>3.7</td>
<td>195</td>
<td>166</td>
<td>305</td>
<td>254</td>
<td>230</td>
</tr>
</tbody>
</table>

P1 = Days 45–46; P2 = Days 58–59; P3 = Days 70–71; P4 = Days 72–73.

Basal LH concentrations were not different between Groups S and NS (P > 0.05). Treatment × implant and treatment × bleeding interactions were tested for mean basal concentrations, but none was significant (P > 0.10).

Although the numbers of LH spikes did not differ in cows in Groups S and NS, the mean concentration of LH released during a spike was higher in cows in Group NS (P < 0.01). There was no effect of prolactin spikes on the incidence of LH spikes; in the absence or presence of prolactin spikes the mean LH spike value was 2.9 ng/ml.

Basal concentrations of prolactin differed significantly for the two groups of cows (P < 0.05); the PRID had no effect on the mean basal prolactin level (P > 0.10). The numbers of prolactin spikes were similar but the peak value was higher in Group S cows (P < 0.05). There was a high correlation between length of anoestrus and the number of prolactin peaks (r = 0.87, P < 0.05). There was also, however, a high negative correlation between length of anoestrus and mean basal LH concentration.

The mean duration of suckling during each bleeding period was 8.9 ± 0.3 min and did not vary significantly (P > 0.05) amongst the four bleeding periods. The mean suckling frequency for the four periods was 9 ± 1 times with no difference amongst periods (P > 0.05).
Text-fig. 2. Plasma LH and prolactin concentrations in (a) suckling and (b) non-suckling cows at various times post partum. Cows 3 and 10 were treated with a PRID from Days 60 to 72. Arrows denote the time when suckling started.
Suckling and hormones in beef cows post partum

Discussion

While it has been known for many years that suckling inhibits ovarian function (Oxenreider, 1968) and lengthens the anoestrous period (Wiltbank & Cook, 1958), it is not yet understood how suckling exerts this effect. Several hypotheses have been offered to explain how suckling retards the occurrence of the first oestrus and ovulation post partum. For example, Kann, Martinet & Schirar (1978) suggested that the hyperprolactinaemia associated with suckling may inhibit ovarian activity in the post-partum ewe. However, the effect of reducing hyperprolactinaemia with bromocriptine did not shorten the anoestrous period in beef cows (Williams & Ray, 1980) and Webb, Lamming, Haynes & Foxcroft (1980) reported no relation between plasma prolactin and length of anoestrus in 4 non-suckling dairy cows bled every 6-h from Days 2 to 32 after calving. In the present study suckling was associated with a high plasma prolactin level and there was a high correlation between the number of prolactin peaks and length of anoestrus. There was also, however, a high negative correlation between anoestrous length and mean basal LH concentration. The raised plasma prolactin or lowered plasma LH could both result in inhibited ovarian function.

The transient elevation (> 1.5–2 ng/ml) in plasma progesterone which lasts 3–5 days and precedes the first oestrus has been reported previously (Pope, Gupta & Munro, 1969; Webb et al., 1980). Such transient peaks in plasma progesterone occurred in 4 of the non-suckling cows (Numbers 7, 9, 10 and 11) but did not occur in the suckling cows. The non-suckling cows also had slightly higher LH levels than did the suckling cows. The fact that progesterone administration was followed by oestrus in 5 of 6 cows overall and 2 of the 3 suckling cows merits further study. Exogenous progesterone is known to block oestrus and ovulation (Ulberg, Christian & Casida, 1951) and to have a negative feedback effect on tonic LH release in the ewe (Karsch, Legan, Hauger & Foster, 1977). In the present experiment, basal and tonic levels of LH were lower during than before PRID treatment. When the PRID was removed there was a marked rise in plasma oestradiol and this sequence resembles what happens during the follicular phase in the normal oestrous cycle. The PRID treatment did not interfere with an existing corpus luteum (Cow 10).

It was possible to examine the effect of stress on prolactin secretion in this experiment. Since the intensive bleeding schedule began 30–60 min after cannulation of the jugular vein, the profiles were examined for high plasma prolactin levels during the early portions of the bleeding schedule. In only one cow (No. 10) did a high plasma prolactin level consistently result from the trauma of cannulation: prolactin concentrations were elevated during all the bleeding periods for approximately 5 h after the start of bleeding.

In conclusion, suckling appears to affect both prolactin and LH secretion in the cow. Whether these changes in secretion are causally involved in suckling-induced anoestrus remains to be demonstrated.

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References


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