Effects of Various Steroid Milieus or Physiological States on Sexual Behavior of Holstein Cows

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ABSTRACT: Two experiments were conducted to determine how steroid milieus and pregnancy affect sexual behavior. Experiment 1 was arranged as a Latin square with five ovariectomized cows and five steroid milieus: no steroid (N; progesterone (P4); estradiol benzoate (EB); P4 + EB; and P4 followed by EB (P4:EB). Progesterone was administered via pessary (2 g of P4) for 5 d and EB was injected (1 mg i.m.) on the day before a test day. On a test day, cows were exposed for four 30-min periods, twice each with a tied or a loose estrual test cow (prepared using P4:EB). Sexual behaviors recorded were attempted mounts, successful mounts, front mounts, stands, head butts, chin rests, and vulvar sniffs. Cows exhibited more \((P < .05)\) sexual behavior during periods with the loose estrual test cow than with the tied estrual test cow. Cows receiving P4 alone ranked lowest among treatments for each behavior, whereas cows receiving EB or P4:EB ranked highest or second-highest. Progesterone prevented stands in cows given P4 + EB, but these cows displayed mounting behaviors similar to those of cows given EB and P4:EB. Cows given P4 + EB were similar to those given N for most behaviors. In Exp. 2, 118 intact, lactating cows were observed in groups of four or five for mounting of estrual test cows during 24, 30-min observation periods on 8 d over 2 yr. The design was an incomplete block with physiological state, parity, estradiol, progesterone, and a calculated estrogen:progesterone ratio included in the model. Each block included one or two cows at 23 ± .8 d after insemination, divided retrospectively into one pregnant and two non-pregnant groups (low [≤ 1 ng/mL] vs high progesterone), and other cows at 89 ± 1.0, 152 ± 1.2, and 234 ± 1.7 d of gestation (six physiological groups). Most cows were observed once, but 27 cows were included twice during 2 yr. Only 60% of the 118 cows made attempted or successful mounts even though estrual test cows were always receptive. Physiological state was not associated with amount of mounting because very active (≥ five attempts) and inactive cows were represented in all physiological groups. The estrogen:progesterone ratio on test day accounted for small, but significant, variation in mounting behavior. For cows observed on two different days, correlations between successive observations were .46 for attempted mounts, .78 for successful mounts, and .71 for total mounts. Cows with high progesterone alone, those with low progesterone and low estradiol, and most pregnant cows showed low mounting activity toward receptive estrual cows.

Key Words: Cattle, Behavior, Estrus, Estrogen, Progesterone, Pregnancy

Introduction

Failure to detect estrus in cattle may be influenced by cows that display weak estrual signs that fail to elicit mounting by herdmates, or by herdmates that do not actively mount overtly estrual animals. Cattle in metestrus, diestrus, or early pregnancy are less active mounters than are those in proestrus or estrus (Mylrea and Beilharz, 1964; Hurnik et al., 1975; Esslemont et al., 1980; Glencross et al., 1981; Alexander et al., 1984; Edgerton et al., 1984; Helmer and Britt, 1985). Active mounting corresponds with physiological states when progesterone concentration is at basal level and estradiol concentration is elevated (Glencross et al., 1973).

Estradiol induces estrus in ovariectomized cattle (Melampy et al., 1957), and passive immunization against estradiol inhibits estrus in estradiol-
SEXUAL BEHAVIOR OF COWS

Table 1. Treatment sequences and concentrations of hormones on test day for cows of Experiment 1

<table>
<thead>
<tr>
<th>Weekday</th>
<th>Control (N)</th>
<th>Progesterone (P4)</th>
<th>Estradiol (EB)</th>
<th>P4 + EB</th>
<th>P4:EB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuesday</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>PES</td>
</tr>
<tr>
<td>Wednesday</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>PES</td>
</tr>
<tr>
<td>Thursday</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>PES</td>
</tr>
<tr>
<td>Friday</td>
<td>-</td>
<td>PES</td>
<td>-</td>
<td>PES</td>
<td>PES</td>
</tr>
<tr>
<td>Saturday</td>
<td>-</td>
<td>PES</td>
<td>-</td>
<td>PES</td>
<td>PES</td>
</tr>
<tr>
<td>Sunday</td>
<td>-</td>
<td>PES</td>
<td>-</td>
<td>PES</td>
<td>-</td>
</tr>
<tr>
<td>Monday</td>
<td>-</td>
<td>PES</td>
<td>EB</td>
<td>PES + EB</td>
<td>EB</td>
</tr>
<tr>
<td>Tuesday (test day)</td>
<td>-</td>
<td>PES</td>
<td>-</td>
<td>PES</td>
<td>-</td>
</tr>
</tbody>
</table>

Test day serum hormones

<table>
<thead>
<tr>
<th>E2, pg/mL</th>
<th>P4, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.6 ± 1.2</td>
<td>4.8 ± 1.1</td>
</tr>
</tbody>
</table>

PES = progesterone-impregnated (2 g) pessary; EB = estradiol benzoate (1 mg, i.m.); E2 = serum estradiol 17β; P4 = serum progesterone; ND = not detectable (< 3 ng/mL).

Materials and Methods

Animals and Treatment Regimens

Experiment 1. Nonlactating Holstein cows were used in an experiment designed as a Latin square with five cows and five steroid milieus. Cows were ovariectomized at least 6 mo before initiation of treatment (Vailes and Britt, 1990). Progesterone-impregnated pessaries (PES; 2 g of progesterone; Britt et al., 1986) and estradiol benzoate (EB; 1 mg i.m.) were used to create the different steroid milieus. The five steroid milieus were control with no steroid (N); PES for 5 d (P4); EB; PES for 5 d plus EB on d 4 (P4 + EB); and PES for 5 d with EB on d 7 (P4:EB). Treatments were given so that the desired steroid milieus occurred in all cows on the same test day (Table 1).

The five test days during the experiment were separated by 28-d intervals. The experimental period was from October to February. Cows were kept along with other cattle on a fescue pasture throughout the experimental period. They were provided with corn silage as needed to maintain body condition and moved from the pasture only to administer treatments and to observe sexual behavior. Observations of sexual behavior began 23 h after appropriate treatment groups received EB.

Two additional ovariectomized cows were treated with P4:EB before each test day and used as estrual recipients (test cows) for evaluation of sexual behaviors of each of the five treated cows. On each test day, steroid-treated cows were moved into a corral adjacent to two test areas (approximately 6 m × 15 m) in a grassy lane. One test cow was tied by halter in one test area and the other test cow was kept loose in the other test area.

The five cows to be observed were released in random pairs from the corral. Each pair was split so that one was observed with the tied test cow and the other with the loose test cow. Observation periods lasted 30 min, and each treated cow was tested individually during four test periods on each test day, twice with the loose cow and twice with the tied cow. Two observers experienced in the detection of estrus were present on each test day. Sexual behaviors were recorded as follows: successful mounts (both front feet off the ground); attempted mounts (upward motion and one front foot off the ground); head butts; chin rests; vulvar sniffs; front mounts; no stands (not receptive to mounts by the loose test cow); and stands (receptive to mounts from the loose test cow). Observa-
Table 2. Distribution of cows by physiological states among 24 observation periods in Experiment 2

<table>
<thead>
<tr>
<th>Physiological states</th>
<th>No. of cows</th>
<th>Day after insemination or day of gestation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of observation periods&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant (P&lt;sub&gt;4&lt;/sub&gt; &lt; 1 ng/mL)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13</td>
<td>22.3 ± 1.1</td>
<td>13</td>
</tr>
<tr>
<td>Nonpregnant (P&lt;sub&gt;4&lt;/sub&gt; ≥ 1 ng/mL)</td>
<td>15</td>
<td>22.1 ± 1.2</td>
<td>13</td>
</tr>
<tr>
<td>Pregnant (23 d)</td>
<td>17</td>
<td>23.3 ± 0.9</td>
<td>15</td>
</tr>
<tr>
<td>Pregnant (48 d)</td>
<td>26</td>
<td>89.1 ± 1.0</td>
<td>24</td>
</tr>
<tr>
<td>Pregnant (152 d)</td>
<td>23</td>
<td>152.0 ± 1.2</td>
<td>23</td>
</tr>
<tr>
<td>Pregnant (234 d)</td>
<td>24</td>
<td>234.3 ± 1.7</td>
<td>24</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± SE.
<sup>b</sup>Various physiological states were represented in 13 to 24 of 24 observation periods (four or five cows for 30 min with estrual test cow). Each observation period included cows of at least four nonestrual physiological states.
<sup>c</sup>Serum progesterone on day of observation.

Blood samples were collected from a coccygeal vessel of each cow at initiation of treatment and on the morning of each test day in Exp. 1. Blood samples were allowed to clot and serum was separated by centrifugation (1,600 x g). Serum was stored at -20°C until it was assayed for estradiol and progesterone by RIA (Cox and Britt, 1982; Archbald et al., 1984). During Exp. 2, single blood samples were collected from each cow on the day of observation. These samples were assayed for progesterone, estradiol, estrone (Frank et al., 1983), and testosterone using RIA (McKinnie et al., 1988).

Across experiments, intraassay and interassay CV were 12 and 13%, respectively, for estradiol and 9 and 12% for progesterone. Intraassay CV were 9% for estrone and 10% for testosterone.

Statistical Analyses

Data from Exp. 1 and 2 were analyzed by least squares analysis of variance using the GLM and CATMOD<sup>®</sup> procedures of SAS (1985). For Exp. 1, each cow was observed twice each with the tied estrual cow and twice each with the loose estrual cow on each of five test days. Therefore, two analyses were run: the first was for a Latin square with cow, test day, and treatment (steroid milieu) in the model to test for treatment effects; the second analysis was a split-plot within the Latin square with main effects as in the first analysis and test cow, test period, and the test cow x test period interaction in the subplot. This second analyses was necessary because of test cow and test period effects. Orthogonal contrasts from the first analyses (treatment effects) were used to compare several behavioral responses among the various treatment groups: control (treatment N) vs
high progesterone (treatment P4); N and P4 vs treatments including estradiol (EB, P4 + EB, P4:EB); P4 vs EB, P4:EB; and P4 + EB vs EB, P4:EB. Also, behavioral means for each treatment group were ranked from highest to lowest (5 to 1) for each of six behavioral traits (excluding front mounts and no stands), and these rankings were subjected to a one-way analysis of variance using treatment as the independent variable. These means were compared using the orthogonal contrasts listed above.

Experiment 2 was conducted as an incomplete block with observation group as the blocking term. For the 118 cows (pregnant or nonpregnant) dependent variables were activity index, attempted mounts, successful mounts, and total mounts (attempted plus successful). Activity index was defined as 1 if a cow made any mounting attempts or 0 if she had none. Independent class variables in the models were observation group, parity (primiparous vs multiparous), and physiological state. Linear effects of estradiol and progesterone and a calculated estrogen:progesterone ratio (ratio = [estradiol + (estrone/200)]/progesterone) were also included in models. Estrone (pg/mL) was discounted because of lower potency compared to estradiol (Emmens, 1962) and to minimize confounding with physiological state because estrone is at much higher concentrations in maternal plasma after the first trimester of pregnancy (Robertson and King, 1979). Analyses were repeated within active cows (any cows having mounting attempts; activity index = 1) for these dependent variables: attempted mounts, successful mounts, and total mounts. A separate analysis using the CATMOD procedure was conducted for the categorical dependent variable, activity index, using physiological state as the independent variable.

For the 27 cows that were included in two different observational periods, the relationship between observations was examined by correlation between successive observations and by regression using mounting data from initial observation periods as independent variables and subsequent data as dependent variables. For these models, an additional class variable was the change in physiological state from the initial to subsequent observation period: 1) similar physiological state in the same year (n = 6); 2) similar physiological state in different years (n = 8); and 3) different physiological states in different years (n = 13). From initial mounting data, these 27 cows were classified as either active (at least one mounting attempt, n = 11) or inactive (n = 16), and this classification was used as an independent variable in a CATMOD procedure with subsequent activity index (1 or 0) as the categorical dependent variable.

Results

Experiment 1

On a test day, cows in treatment N yielded serum with basal concentrations of both estradiol (< 8 pg/mL) and progesterone (< .3 ng/mL); serum from cows in treatment P4 contained high levels of progesterone (> 4 ng/mL) and basal estradiol; serum from cows in treatment EB contained high estradiol (> 30 pg/mL) and basal progesterone; serum from cows in treatment P4 + EB contained high concentrations of both progesterone and estradiol; and serum from cows in treatment P4:EB contained high estradiol and basal progesterone, after priming with progesterone before the test day (Table 1).

Treatment means for activity during 30-min test periods for five sexual behaviors and average rank of those five behaviors plus head butts are shown in Figure 1 (panels A to F). Means for stands include periods only with the loose estrual test cow. Data on head butts are not shown, but the rank of means was similar to that of vulvar sniffs. Cows that had only high progesterone on test day had fewer chin rests and vulvar sniffs than did untreated (N) cows and consistently ranked lowest for all sexual behaviors. Cows in P4 and N groups were less active for most behaviors than cows with high estradiol. For example, more than twice as many successful and attempted mounts were observed in the P4:EB group than in either the N or P4 groups.

Cows that had high levels of progesterone and estradiol simultaneously were not receptive to mounting attempts and had fewer head butts and chin rests than cows that received EB. Both groups had a similar number of attempted and successful mounts. The estrual groups (EB and P4:EB) ranked first and second for each sexual behavior and included cows that consistently stood when mounted by the loose estrual test cow. One N cow did stand four times when mounted and one P4 + EB cow stood once when mounted from the front. Front mounts were initiated only by cows in EB or P4:EB groups (.4 and .2 ± .1 per 30 min, respectively).

Test day had no significant effect on amount of sexual activity and cow affected only the number of chin rests (P < .05); one cow averaged 6.4 chin rests per test period, compared with 2.8 for other cows.

Different test periods within test day did not affect estrous behavior of the treated cows, but access to a loose estrual test cow resulted in greater intensity of behavior than access to a tied
estrual test cow. For example, the loose estrual cow received more \((P < .05)\) successful mounts \((3.2 \pm .5\) vs \(1.5 \pm .2)\), attempted mounts \((2.1 \pm .4\) vs \(1.2 \pm .3)\), and chin rests \((4.5 \pm .7\) vs \(2.5 \pm .4)\) than did the tied cow.

**Experiment 2**

Concentrations of estradiol, estrone, progesterone, and the estrogen:progesterone ratio by physiological states are given in Table 3. Testosterone

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Figure 1. Means for various sexual behaviors (panels A to E) and overall rank of sexual activity (panel F) for cows given progesterone alone (P4), no steroid (N), estradiol benzoate (EB), progesterone plus estradiol benzoate simultaneously (P4 + EB), or progesterone followed by estradiol benzoate (P4:EB). Pooled SE are given for each trait. Orthogonal contrasts that were significant \((P < .01)\) are shown in each panel.
concentrations were below assay sensitivity (3 ng/mL). Progesterone concentrations were elevated in one nonpregnant group by definition and in pregnant groups. Nonpregnant cows with high progesterone and cows pregnant 23 ± .9 and 89 ± 1.0 d had basal levels of estradiol (< 8 pg/mL), and all except one cow (92 d pregnant) had undetectable (< 80 pg/mL) estrone. Nonpregnant cows with low progesterone had nondetectable concentrations of estrone and basal levels of estradiol, except for four cows that had 9.8 to 16.7 pg/mL of estradiol on the day of observation. Estradiol and estrone concentrations were elevated in cows pregnant 234 ± 1.7 d, whereas those steroids were intermediate for cows 152 ± 1.2 d in gestation.

Relationships of physiological states to mounting activity toward estrual test cows are given in Table 4. No differences in mounting were found among cows in the different physiological states or among parities, except that multiparous cows had more attempted mounts (.8 vs .3, P < .05) than did primiparous cows. Inactive and very active (≥ 5 total mounting attempts; n = 19) cows were present in all physiological groups; 60.2% of 118 cows had at least one attempted or successful mount of the estrual test cow.

Pregnant or nonpregnant cows did not stand for an estrual test cow, although 38 cows (32%) were mounted or incompletely mounted by an estrual test cow. Those cows were from each of the physiological groups and most received fewer than five mounting attempts, except for one pregnant cow (236 d) that received 15 mounting attempts in one 30-min period and two other pregnant cows (153 and 157 d) that received six mounting attempts each.

Regression coefficients of the estrogen:progesterone ratio were small but significant (P < .05) for attempted mounts (b = .031), successful mounts (b = .080), and total mounts (b = .110). However, that regression did not account for a significant relationship to the categorical activity index (0 or 1 for mounting behavior; b = .001, P > .8). Regressions involving estradiol or progesterone alone were not significant for any mounting trait. Analyses using only data from active cows gave similar results. The CATMOD procedure also failed to reveal significant effects of physiological state on activity index; 54 to 73% of cows of various physiological states displayed at least one mounting attempt.

For cows with repeated observations, physiological change from one period to the next was not related to subsequent mounting behavior. However, prior mounting behavior was correlated with subsequent behavior for attempted mounts (r = .46, P < .05), successful mounts (r = .78, P < .0001), and total mounts (r = .71, P < .0001). The 11 cows that were active at first observation (one or more mounting attempts) had a numerically higher activity index at the subsequent observation than did the 16 previously inactive cows (.73 vs .44, P = .14).

Of 353 total mounting attempts among cows in 24 observation groups, 96.6% involved the estrual test cow (68.8% on test cows and 27.7% by test cows). Cows other than the estrual test cow were involved together in 12 mounting attempts. In all 12 cases, mounted cows were actively involved with the estrual test cow and mounting cows were involved with the test cow in 11 of 12 cases.

In all 24 observation periods, estrual test cows were mounted with 15 min after onset of the observation period and test cows were receptive to all mounting attempts. Across all 30-min observation periods, 33.0% of successful mounts occurred during the first 5 min and 66.5% of successful mounts occurred during the first 15 min of exposure to estrual test cows.

Discussion

Use of ovariectomized cows in Exp. 1 enabled us to determine how progesterone and estradiol act independently or collectively to modify various sexual behaviors. Progesterone strongly inhibited sexual activity both in comparison to untreated cows (P4 vs N) and to those given EB (P4 + EB vs EB, P4:EB). Our findings are consistent with other studies in which cows in diestrus, having high progesterone and low estradiol, had less mounting activity than cows at other stages of the cycle (Alexander et al., 1984; Edgerton et al., 1984; Helmer and Britt, 1985). Estradiol alone strongly stimulated sexual activity: the EB and P4:EB groups displayed the greatest number of stands and mounts, and showed an increased frequency of secondary estrual behaviors. Similarly, Glenn et al. (1981) observed that 11 different sexual behaviors (both initiating and receiving components) intensified in intact cows that had high blood levels of estradiol and low levels of progesterone.

The overall ranking of sexual behaviors revealed similar sexual behavior between the progesterone-primed estradiol group (P4:EB) and the group given estradiol without previous exposure to progesterone (EB). Some researchers have reported that an appropriate dose of estradiol alone would effectively induce estrus in ovariectomized cows or heifers (Asdell et al., 1945; Ray, 1965; Katz et al., 1980; Cook et al., 1986; Knutson and Allrich, 1987); however, others suggested that priming with progesterone was necessary to initiate estrus using estrogen (Carrick and Shelton, 1969; Murphree and Tsou, 1975) or that priming with
Table 3. Concentrations of estradiol (E₂), estrone (E₁), progesterone (P₄), and estrogen:progesterone ratio (E:P₄) by physiological states of Experiment 2

<table>
<thead>
<tr>
<th>Physiological states</th>
<th>No. of cows</th>
<th>E₂, pg/mL</th>
<th>E₁, pg/mL</th>
<th>P₄, ng/mL</th>
<th>E:P₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant (P₄ &lt; 1 ng/mL)b</td>
<td>13</td>
<td>6.1</td>
<td>NDc</td>
<td>4d</td>
<td>18.7</td>
</tr>
<tr>
<td>Nonpregnant (P₄ ≥ 1 ng/mL)</td>
<td>15</td>
<td>2.2</td>
<td>ND</td>
<td>4.8</td>
<td>7.7</td>
</tr>
<tr>
<td>Pregnant (23 d)</td>
<td>17</td>
<td>2.4</td>
<td>ND</td>
<td>5.1</td>
<td>7.7</td>
</tr>
<tr>
<td>Pregnant (89 d)</td>
<td>26</td>
<td>3.6</td>
<td>3.5e</td>
<td>6.5</td>
<td>6.6</td>
</tr>
<tr>
<td>Pregnant (152 d)</td>
<td>23</td>
<td>12.6</td>
<td>332.0</td>
<td>6.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Pregnant (234 d)</td>
<td>24</td>
<td>34.2</td>
<td>628.3</td>
<td>6.7</td>
<td>6.2</td>
</tr>
<tr>
<td>Pooled SE</td>
<td></td>
<td>1.4</td>
<td>29.7</td>
<td>.4</td>
<td>9.9</td>
</tr>
</tbody>
</table>

*a*E:P₄ calculated as follows: E:P₄ = (E₂ + E₁/200)/P₄. E₁ was divided by 200 to discount high concentrations present only in mid- and late pregnancy.

*bSerum progesterone on day of observation.

*cND = Not detectable (< 80 pg/mL).

*dProgestrone set at .3 ng/mL when below sensitivity of assay.

*eEstrone detected in only 1 of 26 cows.

progesterone had adverse effects on standing behavior (Davidge et al., 1987). Differences between the study of Davidge et al. (1987) and our first experiment included the route of administering progesterone (i.m. vs pessary) and the timing of the estradiol injection relative to serum progesterone concentrations. At the time of estradiol injection and on the day of observation in Exp. 1, concentrations of progesterone in the P₄:EB group were < 1 ng/mL (Britt et al., 1986; Table 1). However, Davidge et al. (1987) reported that cows receiving either 300 or 500 mg of progesterone per day had serum concentrations > 1 ng/mL during and after the time of treatment with estradiol. Therefore, those cows were likely to have been more similar to our P₄ + EB group than to the EB or P₄:EB groups.

High progesterone abolished the standing reflex induced by EB; the P₄ + EB group had only one stand (from a front mount), whereas the other two estradiol groups averaged 3.0 ± .9 stands. Although the contrast P₄ + EB vs N was not tested directly, progesterone and estradiol were antagonistic in the P₄ + EB group such that sexual behavior was similar to that of ovariecotomized cows that received neither steroid. These two groups (N and P₄ + EB) were intermediate in amount of sexual behavior between those cows having only high progesterone and those having only high estradiol on the test day.

Simultaneously high levels of progesterone and estradiol affected some behaviors more than others; standing, butting, and chin resting were inhibited by progesterone, but mounting activity

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Table 4. Least squares means and pooled standard errors for mounting behavior by physiological states in Experiment 2

<table>
<thead>
<tr>
<th>Physiological states</th>
<th>No. of cows</th>
<th>Percentage of cows activeb</th>
<th>No. of attempted mountsc</th>
<th>No. of successful mountsd</th>
<th>Total mountsd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant (P₄ &lt; 1 ng/mL)</td>
<td>13</td>
<td>54</td>
<td>.9</td>
<td>2.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Nonpregnant (P₄ ≥ 1 ng/mL)</td>
<td>15</td>
<td>73</td>
<td>.5</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Pregnant (23 d)</td>
<td>17</td>
<td>65</td>
<td>.4</td>
<td>1.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Pregnant (89 d)</td>
<td>26</td>
<td>62</td>
<td>.4</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Pregnant (152 d)</td>
<td>23</td>
<td>61</td>
<td>.8</td>
<td>1.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Pregnant (234 d)</td>
<td>24</td>
<td>50</td>
<td>.8</td>
<td>1.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Overall mean (n = 118)</td>
<td>–</td>
<td>60</td>
<td>.6</td>
<td>1.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>–</td>
<td>11</td>
<td>.3</td>
<td>.5</td>
<td>.7</td>
</tr>
</tbody>
</table>

*a*Differences among physiological states were not significant for any variable.

*bPercentage of cows that had any successful or attempted mounts.

*cIncludes only attempts or mounts toward the estrual test cow.

*dIncludes all mounts and mounting attempts by nonestrual cows including 12 attempts not directed toward the estrual test cow. Due to those attempts and rounding errors, these values are not a simple total of attempted and successful mounts.
was not different among the P4 + EB, EB, or P4:EB groups. Davidge et al. (1987) also found that standing behavior was inhibited more by progesterone than was mounting behavior. There may be a threshold level of progesterone or a ratio of progesterone to estrogen above which standing does not occur. Ovulations with undetected estruses were associated with higher concentrations of progesterone in milk fat at estimated time of ovulation (Schopper and Claus, 1986). Glencross (1987) induced follicular growth and associated elevated estradiol in diestrous heifers using pulsatile doses of GnRH, yet heifers would not stand until progesterone declined to basal concentrations.

Steroid concentrations in ovariectomized cows of Exp. 1 can be theoretically related to what we would expect in intact cows as follows: treatment N corresponds with acyclic postpartum cows or cows in metestrus; treatment P4 is equivalent to cows during diestrus or during early pregnancy, treatment P4 + EB is most like that of cows during late gestation; treatment EB corresponds to cows at the start of the first cycle postpartum, when a rise in progesterone does not usually precede the increase in estradiol; and treatment P4:EB is most like an estrual cow, in which an increase in progesterone precedes the rise in estradiol (Glencross et al., 1973; Walters et al., 1984; Schallenberger et al., 1985a,b; Meisterling and Dailey, 1987; Eissa and El-Belely, 1990). Hormonal concentrations of cows in Exp. 2 (Table 3) were consistent with those categories. Of 13 nonpregnant cows with low progesterone, nine cows also had low estradiol (likely metestrus), similar to the N group of Exp. 1, and the other four cows were likely in early proestrus. Other cows in Exp. 2 fit into either the P4 classification (nonpregnant, high progesterone; pregnant 23 ± .8 or 89 ± 1.0 d) or P4 + EB (pregnant 234 ± 1.7 d), except for cows at 152 ± 1.2 d of pregnancy, which were intermediate between P4 and P4 + EB because of lower estrogen concentrations.

Based on those theoretical physiological categories and the results of Exp. 1, the sexual activity of intact cows should rank as follows: cows in diestrus or early pregnancy < noncyclic postpartum or metestrus cows < cows in late gestation < first-cycle-postpartum estrual cows ≤ estrual cows. Using this reasoning, we expected cows in mid- and late gestation in Exp. 2 to be more active mounters than either cows in early gestation or nonpregnant cows with high progesterone on day of observation. This postulation was not supported by results of Exp. 2, even when inactive cows were omitted.

Why cows in Exp. 2 did not respond as predicted from results of Exp. 1 may be due to differences in methods of exposure of cows to estrual test cows or to the acute vs chronic nature of steroid milieus vs natural physiological states. In Exp. 1, cows were exposed individually to receptive estrual cows, thus minimizing social inhibitions that may be present in group situations, as in Exp. 2 or in typical herd environments. Also, in Exp. 1, progesterone was administered to ovariectomized cows for only 5 d and estradiol was given as a single injection, compared with chronically elevated progesterone for all pregnant cows and chronically elevated estradiol and estrone for cows in mid- and late gestation in Exp. 2. Exposure to elevated levels of these steroids for long periods of time may affect responses of some cows to the stimulus of an estrual cow. Alternatively, other hormones (e.g., cortisol; Cook et al., 1987) or factors associated with lactation may affect mounting behavior of cows beyond that predicted by estradiol and progesterone.

Of particular interest in Exp. 2 were the high correlations between subsequent observations for the same cows over time periods up to 1 yr, suggesting that the sample population included cows that were usually active mounters and others that were not, regardless of physiological state. Asdell et al. (1945) observed that intensity of sexual activity before ovariectomy was generally inversely related to the amount of estradiol needed to stimulate estrus after ovariectomy. This would suggest physiological differences among individual cows in expression of estrus. Similarly, the current study very clearly demonstrated mounting differences among individual cows. Estrual test cows were mounted (attempted + successful mounts) as few as once and as many as 26 times in different 30-min observation periods in Exp. 2. This variation was not related to receptivity of a particular test cow. The receptivity of estrual test cows was further tested by observing them individually with other estrual cows on nine occasions on 7 d during Exp. 2. All nine estrual cows mounted the respective estrual test cows and averaged 4.3 times as many total attempts (9.1 ± 1.0) as the 118 nonestrual cows included in Exp. 2. The fact that estrual test cows were always receptive to any mounting attempts emphasizes the importance of having actively mounting cows for efficient detection of estrus in herds.

Although none of the cows in Exp. 2 was receptive to mounting attempts from an estrual test cow, it was evident that some pregnant cows were particularly sexually attractive to the test cows. Such cows might be receptive to being mounted at some time during pregnancy. Those cows did not have unusual hormonal profiles, which is consistent with the report of Thomas and Dobson (1989) that clearly documented receptive
behavior among pregnant cows. They also reported that such behavior occurred more often after 120 d of gestation and that it could occur more than once during a pregnancy or during successive pregnancies, which is consistent with the repeatability we observed for mounting behavior.

During both experiments, observations were for short durations (30 min) during daylight hours away from feeding and milking areas. It is probable that patterns of mounting behavior would be quite different under herd situations (Hurnik et al., 1975), and possibly more cows would have been active in Exp. 2 if exposed for longer periods. However, the effect of introduction of animals (Alexander et al., 1984) as we did in these experiments allows for concentrated expression of mounting behavior. Higher proportions of mounting behavior were observed during the first few minutes of this study and also in a prior study (Bitt et al., 1986). Such intensive mounting periods would be less likely within a herd. Also, Helmer and Bitt (1985) reported no diurnal pattern of mounting behavior among heifers. In herds with long calving and breeding periods, the formation of large, sexually active groups (Kilgour and Dalton, 1985) is less likely, and it has been our experience that some cows may go through an entire estrual period without much interest from nonestrual herdmates.

In conclusion, our data clearly indicate effects of exogenous progesterone and estradiol on expression of sexual behavior in ovariectomized, nonlactating cows. However, the relationship of those hormones to mounting behavior among intact, nonestrual or pregnant, lactating cows was not as evident. Differences among individual cows in willingness to mount estrual cows are real and seem to be more complex than simple relationships of progesterone and estradiol.

Implications

Progesterone alone inhibited estrous behavior and estradiol alone stimulated estrous activity in ovariectomized cows. Estradiol and progesterone together, however, resulted in intermediate sexual behavior, with standing being affected more negatively than mounting. In contrast, physiological states of nonestrual, intact cows were not clearly associated with mounting behavior. However, a few individual cows of various physiological states were quite active mounters. Cows with high progesterone alone, those with low estrogen and low progesterone, and most pregnant cows had low mounting activity. For efficient detection of estrus in herds, cows will need to be managed to ensure presence of animals that actively mount estrual cows.

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