Acute and Chronic Changes in Luteinizing Hormone Secretion and Postpartum Interval to Estrus in First-Calf Suckled Beef Cows Exposed Continuously or Intermittently to Mature Bulls[1,2]

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ABSTRACT: The objective of this study was to determine whether patterns of LH secretion are acutely or chronically affected by the presence of mature bulls in postpartum first-calf suckled beef cows exposed to bulls either continuously or intermittently beginning on d 30 after birth. Crossbred cows were assigned randomly to be either continuously exposed to (BE; n = 20) or isolated from bulls (NE; n = 32) at calving, or exposed continuously (NEBE; n = 10) or intermittently (BEI; n = 21) to bulls beginning on d 30. The BEI cows were exposed to bulls for 2 h every 3rd d for 18 d. Ten cows from the NEBE, BEI, and NE treatments only were fitted with indwelling jugular catheters. Intensive blood sampling of NEBE and BEI cows began within 45 min after they were placed with bulls for 2 h on d 30, and for BEI cows on each d after 2 h of exposure. Samples were collected at 15-min intervals for 6 h beginning on d 30 for NEBE, BEI, and NE cows; sampling continued at 3-d intervals until d 48. Samples were assayed for LH by RIA. Cows were observed twice daily (am:pm) for estrus. More (P = .07) BE and NEBE (75%) cows showed estrus by the end of the study than BEI and NE cows (48%). Interval to estrus was longer (P < .05) in BEI and NE cows (95.6 ± 6.1 d) than in BE and NEBE cows (75.9 ± 6.1 d). Baseline LH and amplitude and interpeak interval of LH peaks during the first 6 h after 2 h of bull exposure did not differ (P > .10) among treatments on d 30 after birth. However, mean LH and LH pulse frequency were higher (P = .06) for NEBE and BEI cows than in NE cows. Baseline LH and amplitude and duration of LH peaks did not differ (P > .10) over the seven sampling periods among NEBE, BEI, or NE cows. Mean LH and LH pulse frequency were higher (P < .05) in NEBE and BEI cows than in NE cows on each sampling period. Exposing first-calf suckled beef cows to bulls on d 30 after birth increased mean LH concentrations by increasing pulse frequency within a short period after a 2-h exposure. Thereafter, mean LH concentrations were higher in cows that were either continuously or intermittently exposed to bulls. Although mean LH and LH pulse frequency in NEBE and BEI cows were similar, intervals to estrus after bull exposure differed between treatments. The mechanism whereby bulls alter postpartum interval to estrus seems to involve other factors that may be related to but not directly linked with LH secretion.

Key Words: Bovine, Postpartum Interval, LH, Stimulation

J. Anim. Sci. 1996. 74:1098–1103

Introduction

Failure of females to rebreed after calving decreases reproductive efficiency in beef cattle production. One of the most important reasons that cows fail to rebreed, especially first-calf suckled cows, is long postpartum periods of anestrus (Wiltbank, 1970). Many factors affect the length of the postpartum anestrus interval in beef cows (Randel, 1990; Short et al., 1990; Williams, 1990). One of these, exposure to mature bulls, shortens the postpartum interval to resumption of ovarian cycling activity in suckled multiparous and primiparous cows (Zalesky et al., 1984; Custer et al., 1990; Fernandez et al., 1993). The physiological interactions that induce this response are complex and remain unclear (Custer et al., 1990).

[1]This research was supported by, and is contribution no. J 4044 from, the Montana Agric. Exp. Sta. This is a contributing project to the Western Regional Research Project W-112, Reproductive Performance in Domestic Ruminants.

[2]The authors express their gratitude to Aaron Amidon, Mark Andersen, Jeff Anderson, Kory Hofland, Vaughn Holtz, and Dale Reynolds for their invaluable assistance during the course of this study.

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[5]U.S. Dept. of Agric., Agric. Res. Serv., Northern Plains Area, is an Equal Opportunity/Affirmative Action employer and all Agency Services are available without discrimination.

Received September 25, 1995.
Accepted January 30, 1996.

Introduction of a male to females has been shown to induce a rapid release of LH, mating behavior, and(or) ovulation in mice (Bronson and Desjardins, 1974), sheep (Chesworth and Tait, 1974; Knight et al., 1978; Martin et al., 1980; Poindron et al., 1980; Cohen-Tannoudji and Signoret, 1987), and goats (Chemineau et al., 1986; Claus et al., 1990). If the LH response to bulls is as rapid as that observed in other species, then the experiment conducted by Custer et al. (1990) to answer the question of LH involvement in the bull effect was not designed to detect such a rapid response because sequential samples were taken initially 1 wk after introduction of bulls and collected only once weekly until estrus. However, the results of Baruah and Kanchev (1993) seem to support the hypothesis that bull exposure induces a rapid change in LH concentrations in suckled cows.

The objectives of this experiment were to determine whether exposure to bulls continuously beginning on d 30 after birth or for 2 h every 3rd d beginning on d 30 after birth alters the postpartum interval to first estrus and acute or chronic patterns of LH concentrations in first-calf suckled beef cows.

Materials and Methods

Eighty-three first-calf, crossbred Hereford × Angus and Angus × Hereford, suckled beef cows maintained at the Bozeman Livestock Center, Montana State University, were assigned to one of two treatments within 72 h of calving: 1) exposure to mature epididectomized bulls (**BE**; n = 20) or 2) isolation from bulls (**NE**; n = 63). Body weight and condition score (9 = obese; 1 = emaciated) for each cow were obtained within 72 h of calving and at 28-d intervals until estrus. Average body weight and condition score of cows after calving were 450 ± 41 kg and 5.6 ± .5, respectively. Cows calved from January 30 to March 13, 1992, and the average calving date was February 19. The experiment ended June 1, 1992, at the start of the breeding season.

On d 30 after birth, NE cows were assigned either to remain in their initial treatment (NE, n = 32) or they were assigned to continuous exposure to bulls (**NEBE**, n = 10) or intermittent bull exposure (**BEI**, n = 21). Cows in the BEI treatment were placed in a pen approximately 8 m wide × 25 m long and exposed to a sterile, mature bull for 2 h on d 30 after birth and on every 3rd d thereafter until d 48 after birth. The BEI cows were placed in a separate pasture from the pastures containing either the BE or NE cows after their first exposure to a bull. The pasture containing the BEI cows was separated from the BE pasture by approximately .7 km and was adjacent to, but not visible from, the NE pasture. Cows assigned to the NEBE treatment were transferred in a trailer from the NE pasture to the BE pasture. The bull:cow ratio was 1:15. Cows were fed a medium-quality mixed-grass and legume hay until pasture grasses became available and were provided free access to mineralized salt and water.

Cows were observed twice daily (between 0600 and 0800 and 1700 and 1900) for 40 min for behavioral estrus. Only those cows that stood to be mounted by a herdmate or by a sterile bull were considered to be in estrus.

Intensive Blood Sampling for Luteinizing Hormone. Two d before the initial bleeding, each of 10 cows from each treatment was fitted surgically with an indwelling jugular catheter (Custer, 1988). Cows were chosen from each treatment to be bled by calving date so that calving dates of three cows, one each from the NEBE, BEI, and NE treatments, were separated by no more than 3 d. Beginning on d 30 after birth, cows in the BEI treatment were placed into separate stalls for blood sample collection immediately after they had been exposed for 2 h to a mature, sterile bull. The NEBE cows were treated in the same manner on the following day and then placed into the pasture containing the BE cows. On the 3rd d, NE cows were placed immediately into separate stalls for blood sample collection. Blood samples were collected on this rotation until d 48 after birth. Calves were placed in the stalls with their dams and could suckle ad libitum. Blood samples were collected every 15 min for the next 6 h. Catheters were flushed between samples with 10 mL of sterile, heparinized saline. Blood samples were placed on ice, allowed to clot, then centrifuged at 1,850 × *g* for 30 min. Serum was stored at –20°C until it could be assayed for LH.

Hormone Assays. Concentrations of LH were quantified using the double antibody RIA method of Custer et al. (1990) using bLH-I-5 as the iodinated hormone and standard. The sensitivity of this assay was .11 ng/mL and the inter- and intraassay CV were < 10 and < 15%, respectively.

Statistical Analysis. The proportion of cows that displayed estrus before the beginning of the breeding season was analyzed by chi-square analysis (Lund, 1991). Postpartum interval to estrus for cows that were not observed in estrus by the end of the experiment was calculated by assuming that estrus occurred on the last day of the experiment and subtracting this day from their calving date. These data were then included in the data set for cows that were actually observed in estrus (adjusted interval). Body weight change, body condition change, and postpartum intervals to estrus (adjusted and nonadjusted) were analyzed by separate analyses of variance for a completely randomized design using the GLM procedure of SAS (SAS, 1987).

Mean, baseline, frequency, amplitude, and interpeak interval for LH pulses were generated using the PULSAR (Merriam and Wachter, 1982) program. Data collected from NEBE and BEI cows on d 30 after birth, within 6 h after 2 h of bull exposure were pooled and analyzed by an analysis of variance for a

Table 1. Least squares means for changes in body weight (BW) and condition score (CS) change from day 3 after calving to the end of the experiment for first-calf suckled beef cows either continuously exposed to bulls after calving (BE), continuously exposed to bulls after day 30 after birth (NEBE), intermittently exposed to bulls after day 30 (BEI), or isolated from bulls after calving (NE)

Treatment	n	BW change, kg	CS change
BE	20	37	.18
BEI	21	45	.21
NE	32	28	.18
NEBE	10	26	.17
SEM[b]	—	10.2	.11

[a]Pooled standard error of the mean; df = 79.

Table 2. Percentages exhibiting estrus and intervals to estrus for first-calf suckled beef cows either continuously exposed to bulls after calving (BE), continuously exposed to bulls after day 30 after birth (NEBE), intermittently exposed to bulls after day 30 (BEI), or isolated from bulls after calving (NE)

Treatment	n	Exhibiting estrus, %	Postpartum interval to estrus, d	Postpartum interval to estrus, d[a]
BE	20	80[b]	62.6[b]	71.3[b]
BEI	21	38[d]	89.8[c]	101.6[d]
NE	32	58[cd]	78.5[c]	89.3[c]
NEBE	10	70[bc]	64.4[b]	77.8[b]
SEM	—	—	17.3[e]	33.1[f]

[a]Adjusted to last day of experiment; assumes that cows that failed to show estrus by end of experiment did so on last day and calculated by subtracting last day of experiment from calving date.

[b,c,d]Means within a column lacking common superscript letters differ ($P < .05$).

[e]df = 46.

[f]df = 79.

completely randomized design using the GLM procedure of SAS (SAS, 1987). Data for these characteristics over the six sampling periods were analyzed by an analysis of variance for a completely randomized design using the GLM procedure of SAS (SAS, 1987) with the repeated measures option with day as the repeated measure. One cow each in both the NE and NEBE treatments had what appeared to be a preovulatory-like surge of LH during the course of the experiment, and data for these cows from the day of the surge onward were excluded from any further analysis.

Means for body weight and body condition score change from d 3 after calving to estrus, postpartum interval to estrus, mean LH concentrations, and LH pulse frequencies were compared using orthogonal contrasts (SAS, 1987). Specific contrasts for body weight change were BEI vs BE, NEBE, NE; NEBE vs BE, NE; and BE vs NE. Specific contrasts for postpartum interval to estrus were BEI vs BE, NEBE, NE; BEI vs BE, NEBE; and BEI vs NEBE. Specific contrasts for characteristics of patterns of LH concentrations on d 30 were NE vs NEBE plus BEI, and for sampling periods from d 30 to 48 were BEI vs NEBE, NE and BEI vs NEBE.

Results

Body weights and condition scores of cows within treatments increased over the course of the experiment (Table 1). Neither body weight nor condition score change differed ($P > .10$) among treatments (Table 1).

The proportion of cows exhibiting estrus by the end of the experiment did not differ ($P > .10$) between cows in the BE and NEBE treatments (Table 2). More ($P < .05$) BE and NEBE cows exhibited estrus by the end of the experiment than BEI cows (Table 2). The proportion of NEBE cows that displayed estrus by the end of the experiment did not differ ($P > .10$) from that of NE cows (Table 2), and the proportion of BEI cows that showed estrus by the end of the experiment did not differ ($P > .10$) from that of NE cows (Table 2).

Postpartum interval to estrus, expressed on the basis of only those cows that were observed in estrus by the end of the experiment, did not differ ($P > .10$) between BE and NEBE cows (Table 2) or between cows in the BEI and NE treatments ($P > .10$; Table 2). However, postpartum interval to estrus was shorter ($P < .05$) for BE and NEBE cows than for either BEI or NE cows (Table 2). Adjusted postpartum interval to estrus (analysis that included all experimental units) did not differ ($P > .10$) between BE and NEBE cows but was shorter ($P < .05$) than those for BEI and NE cows (Table 2). Adjusted postpartum interval to estrus was longer ($P < .05$) for BEI cows than for NE cows (Table 2).

Baseline LH, amplitude of LH peaks, and interpeak interval of LH peaks for patterns of LH concentrations did not differ ($P > .10$) among treatments during the 6-h period after 2 h of bull exposure on d 30 after birth. However, mean LH concentrations and LH pulse frequency were higher ($P = .06$) for cows exposed to bulls (BEI + NEBE than for NE cows; Table 3).

There was no ($P > .10$) treatment or treatment × sampling period interaction for baseline LH, amplitude of LH peaks, or interpeak interval for patterns of LH concentrations from d 30 to d 48 after birth. Cows isolated from bulls (NE) had lower ($P = .05$) mean LH concentrations and LH pulse frequencies over the sampling periods than either NEBE or BEI cows (Table 4).

Table 3. Least squares means of mean LH concentrations and LH pulse frequency for first-calf suckled beef cows during a 6-hour interval after 2 hours of either bulls exposure (NEBE + BEI) or sham exposure (NE) on day 30 after birth

Treatment	n	Mean LH, ng/mL	LH pulse frequency, pulses/h
NEBE + BEI	20	1.41[a]	1.10[a]
NE	9	1.24[b]	.71[b]
SEM	—	.25[c]	.45[d]

[a,b]Means within columns lacking common superscript letters differ (P = .06).
[c,d]df = 26.

Table 4. Least squares means for mean LH concentrations and LH pulse frequency for 6 sampling periods from day 30 to 45 after birth for first-calf suckled beef cows either continuously exposed to bulls after day 30 after birth (NEBE), intermittently (2 hours every 3rd day) exposed to bulls after day 30 (BEI), or isolated from bulls after calving (NE)

Treatment	n	Mean LH, ng/mL	LH pulse frequency pulses/h
NEBE	9	1.47[a]	.91[a]
BEI	10	1.53[a]	1.12[c]
NE	9	1.24[b]	.82[b]
SEM	—	.15[c]	.39[c]

[a,b]Means within a column lacking common superscript letters differ (P = .05).
[c]df = 117.

Discussion

Exposing postpartum first-calf suckled beef cows continuously to mature bulls (BE) within 72 h of calving or after d 30 after birth (NEBE) increased the proportion of cows that exhibited estrus by the beginning of the breeding season and reduced the postpartum interval to estrus by an average of 15 d compared to isolating cows from bulls (NE) . This result confirms results obtained in our laboratory in past experiments for first-calf suckled beef cows (Custer et al., 1990; Fernandez et al., 1993) and supports the conclusion that exposing suckled *Bos taurus* cows to bulls decreases the postpartum interval to resumption of ovarian cycling and breeding activities (MacMillan et al., 1979; Zalesky et al., 1984; Alberio et al., 1987).

In the present study we evaluated whether the postpartum interval to estrus could be altered in cows exposed intermittently to mature bulls (BEI) for 2-h intervals every 3rd d between d 30 and 48 after birth (18 d). We found that this type of exposure did not alter the proportion of cows that exhibited estrus by the beginning of the breeding season or reduce the postpartum interval to estrus in first-calf suckled cows. This result seemed to be independent of any apparent nutritional effect because BEI cows gained weight and body condition throughout the experimental period to the same extent as cows in the other treatments.

In contrast to this result, Petropavlovskii and Rykova (1961) reported that exposure of early postpartum cows to a vasectomized bull for 3 to 4 h twice daily decreased postpartum interval to conception compared to isolation of cows from the bull. Two experimental differences are obvious between our study and that of Petropavlovskii and Rykova (1961): 1) cows were exposed intermittently after d 30 after birth in the present study, and 2) duration and period of exposure differed. One might conclude that in order to obtain the biostimulatory effect of the bull, the duration of exposure must be greater than 2 h daily and(or) the frequency or intensity of exposure must be greater than six times over an 18-d period.

The postpartum anestrus interval to estrus in suckled cows is characterized by low systemic mean concentrations of LH caused by low-frequency pulses in the pattern of LH secretion by the pituitary gland (for review see Short et al., 1990). As the interval from calving to estrus increases in suckled cows, mean concentrations of LH increase as a result of an increase in episodic pulse release of LH (Humphrey et al., 1983) in response to hypothalamic GnRH release (for review see Short et al., 1990). Presumably, changes in mean LH and LH pulse frequency induce the appropriate ovarian changes and ultimately result in estrus and ovulation.

One hypothesis for the biostimulatory effect of bulls on reducing the postpartum interval to estrus in cows is that the presence of bulls should, in some way, alter the temporal changes in mean LH concentrations and(or) episodic release of LH. However, in the study of Custer et al. (1990) the presence of bulls did not alter the temporal patterns of LH secretion compared to isolation from bulls. A major drawback in the design of the study by Custer et al. (1990) for defining the patterns of LH concentrations after bull exposure was the sampling regimen: sequential samples were taken initially 1 wk after introduction of bulls and collected once weekly until estrus. Acute and chronic changes in LH patterns could have been missed. In the present study we examined the acute effect of bulls on LH patterns on d 30 after birth. We found that first-calf suckled cows exposed to bulls for 2 h (NEBE and BEI) had increased mean LH concentrations and LH pulse frequency over the next 6 h compared to sham-exposed cows (NE). This result is supported by the data of Baruah and Kanchev (1993), who reported that oronasal treatment of postpartum lactating dairy cows with bull urine resulted in increased LH concentrations within 80 min. These data indicate clearly that the presence of bulls acutely affects the hypothalamic-pituitary axis regulating LH release in postpartum lactating dairy cows and first-calf suckled beef cows and support the hypothesis that the acute effect of the bull is pheromonally mediated via an olfactory pathway. These results are consistent with the acute effects of males on LH release in female mice (Bronson and Desjardins, 1974), sheep (Chesworth and Tait, 1974; Knight et al., 1978; Martin et al., 1980; Poindron et al., 1980; Oldham and Pearce, 1984; Cohen-Tannoudji and Signoret, 1987), and goats (Chemineau et al., 1986; Claus et al., 1990).

Chronic patterns of LH concentrations were not affected by the time × treatment interaction. However, mean LH concentrations and LH pulse frequencies were higher in cows continuously (NEBE) or intermittently (BEI) exposed to bulls after d 30 after birth than in cows isolated from bulls (NE) during the seven sampling periods (once every 3 d from d 30 to d 48 after birth). This result differs from that of Custer et al. (1990), who reported that the change in mean LH concentrations and LH pulse frequency did not differ between cows continuously exposed to or isolated from bulls within 72 h after calving. As mentioned previously, sampling times, experimental design, and statistical analyses differed between these studies and may account for this discrepancy. Custer et al. (1990) found that mean LH concentrations and LH pulse frequency increased before cows showed estrus, independent of treatment. We could not confirm that result because our sampling period was limited to the first 18 d after bull exposure.

Based on these data and the notion that increased LH secretion plays a significant role in the resumption of ovarian cycling activity, one might conclude that bulls effect a reduction in postpartum interval to estrus by increasing LH secretion acutely and chronically. However, cows that were intermittently exposed to bulls did not exhibit estrus any sooner than cows isolated from bulls. One interpretation of these results is that immediate exposure to bulls induces a pheromonally activated trigger (“signaling type”) that induces a hypothalamic release of GnRH and subsequent acute release of LH, specifically an increase in frequency. This type of stimulation does not result in induction of ovarian cycling activity. However, the continuous presence of the bull carries not only the signaling trigger but other cues or stimuli that induces the early onset of ovarian cycling activity. The nature of this(these) stimuli is not known but may be related to priming pheromones or other exteroceptive cues involved with male-female interactions in cattle. Recently, Wright et al. (1994) reported that treatment of postpartum anestrous cows with cervical mucus from estrous cows reduced the postpartum interval to estrus without affecting mean LH concentrations or frequency and amplitude of LH pulses. They speculated that this effect may be related to pheromones in cervical mucus that may be similar to pheromones produced by bulls.

Implications

Exposing first-calf cows to mature bulls for 2 h on d 30 seems to induce a physiological change in the hypothalamo-hypophyseal axis that results in an increase in concentrations of luteinizing hormone (LH). The stimulus(i) for this physiological effect may be related to pheromonal cues. Continuous or intermittent exposure maintains elevated LH concentrations presumably by altering release of gonadotropin-releasing hormone, but the mechanism for this response is not clear. However, the physiological action of the bull to increase LH frequency and concentrations does not seem to be a major factor in reducing the postpartum anestrus interval in first-calf cows because even in the face of elevated LH concentrations and LH pulse frequency interval to estrus was not reduced in intermittently exposed cows as it was in cows continuously exposed to bulls. Exteroceptive cues other than pheromones may be involved in the biostimulatory effect of bulls on postpartum cows.

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