

Effect of bull urine exposure on postpartum anestrus and breeding performance of first-calf suckled beef cows

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Abstract

The objectives of this experiment was to determine if continuous exposure to bull urine alters resumption of ovarian cycling activity or breeding performance of first-calf suckled beef cows. The null hypotheses were that: 1) interval from exposure to urine to resumption of ovarian cycling activity; 2) proportions of cows cycling at the end of the exposure period; and 3) AI pregnancy rates after estrous synchronization (ES) do not differ between cows exposed to mature bull urine or steer urine. Thirty-eight first-calf suckled beef cows, 4 mature AXH bulls and 4 ten-mo-old AXH steers were used in this study. Cows were stratified by calving date, cow BW, calf BW, calf sex, dystocia score, and BCS, and fitted with a controlled urine delivery device (CUDD) 2 wk before the start of treatments. Cows were assigned randomly to be exposed continuously to urine of bulls (BUE) or steers (SUE) beginning 40 d after calving. Urine was collected from bulls and steers every third d of the experiment. Blood samples were collected from cows starting on d 0 and every third d thereafter until the start of the ES protocol. Likewise, CUDD were filled and refilled on the same schedule. Serum was assayed for progesterone by RIA. A rise in progesterone concentrations of > 0.5 ng/mL in 3 consecutive samples was used to determine resumption of cycling activity. Each cow received a CIDR on d -10 (d 0 = d of TAI) and was given PGF_{2α} (PG) 7 d later at CIDR removal. Cows detected in estrus 60 h after PG and were bred by AI 12 h later; cows not detected in estrus by 60 h after PG received GnRH, and TAI at 72 h after PG. Pregnancy rates were determined 35 d after AI by ultrasonography. Neither interval from urine exposure to resumption of cycling activity nor proportions of cows cycling before the breeding season differed between BUE and SUE cows. However, AI pregnancy rate was greater ($P < 0.05$) for BUE cows than SUE cows. We conclude that continuous exposure to mature bull urine does not affect resumption of ovarian cycling activity but appeared to improve breeding performance of first-calf suckled beef cows.

Introduction

The bull biostimulatory effect reduces postpartum anestrus in first-calf suckled beef cows (Custer et al., 1990; Fernandez et al., 1993, 1996). Little is known of the biological mechanism by which the biostimulatory effect initiates the resumption of ovarian cycling activity in postpartum cows. Recent studies indicate that biostimulation is not mediated through social interactions between bulls and cows (Berardinelli et al., 2004), but rather is mediated through the excretory products of bulls (Joshi et al., 2002). These results indicated that bull biostimulation utilizes a pheromone(s) to stimulate resumption of ovarian cycling activity in beef cows.

Pheromones are small airborne chemicals present in the urine, feces or cutaneous glands and perceived by the olfactory or respiratory systems which cause behavioral and endocrine responses in conspecifics (Rekwot et al., 2000). Oronasally administered bull urine to cows on d 7 postpartum

increased mean LH and FSH serum concentrations within 80 min after exposure (Baruah and Kanchev, 1993). Joshi et al., (2002) showed that cows exposed to the excretory products of bulls resumed ovarian cycling activity earlier after calving than cows not exposed to bulls. It appears that bulls excrete a pheromone into the urine, feces or cutaneous glands that may initiate a neuro-endocrine cascade in cows which results in the resumption of ovarian cycling activity.

The objectives of this experiment were to determine if bull urine exposure alters the resumption of ovarian cycling activity before the breeding season and breeding performance of first-calf beef cows. We tested the hypotheses that: 1) the interval from exposure to resumption of cycling activity; 2) proportion of cows cycling before the breeding season; and 3) AI pregnancy rates, did not differ between cows exposed to mature bull urine or cows exposed to steer urine.

Materials and Methods

Animals and Treatments

Thirty-eight two-yr-old Angus x Hereford first-calf suckled beef cows, four Angus x Hereford epididectomized bulls, and four 1-yr-old Angus x Hereford steers were used in this experiment conducted at the Montana State University Livestock Teaching and Research Center, Bozeman. Animal care, handling, and protocols used in this experiment were approved by the Montana State University Institutional Animal Care and Use Committee.

Cows and calves had no contact with bulls or steers throughout the experiment and had no contact with their excretory products from calving until the start of treatment. Average calving date was Feb. 9. Two wk before the start of treatment cows were stratified by calving date, cow BW, calf BW, calf sex ratio, dystocia score, and BCS and fitted with a controlled urine delivery device (CUDD). Cows were assigned randomly to exposure to steer urine (SUE) or exposure to bull urine (BUE). The average length of exposure time before the start of estrous synchronization was 64 d.

Lot Areas

Two lots were used for this experiment, designated north and south by their geographic location. Each lot contained four pens (41 m x 18 m) that were identical in east-west configuration, bunk space, aspect, slope, and connection to open-shed shelters. Lots were approximately 0.35 km apart. Animals housed in one lot were not able to see or smell animals housed in the other lot; however, there was a possibility that sounds made by animals in one lot could be heard by animals in the other lot. Cows exposed to bull urine (BUE) were housed in the two eastern-most pens the north lot and cows exposed to steer urine (SUE) were housed in the two western-most pens of the south lot.

Urine exposure

Urine was collected from bulls and steers every 3 d throughout the experiment. Cows were continuously exposed to urine using a Controlled Urine Delivery Device (CUDD). Two wk before the start of treatment CUDD were attached to the mid-line of the dulp on the neck, 7.5 cm anterior to the sternum of cows. Laboratory tests using distilled water as the media showed that CUDD

released fluid into the air over a period of 3 d. Therefore, every third day from the start of exposure each CUDD was filled with urine, inspected for non-functioning parts, and repaired.

Nutrition

Cows had free access to good quality, chopped mixed-grass alfalfa hay, and any pasture grasses that were available before the start of the experiment. Once cows and calves were moved into pens they were given free access to the same hay, 0.5 kg•hd⁻¹•d⁻¹ cracked barley, water, and a trace mineral-salt supplement. The TDN of the diet exceeded the NRC requirement for lactating beef cows with a mature weight of 545 kg by approximately 18% (NRC, 1996). Bulls had ad libitum access to fair quality, chopped barley hay. During collection periods bulls were fed 0.5 kg of cracked barley and good quality, chopped mixed-grass alfalfa hay. Steers were fed a finishing ration that consisted of 70% concentrate and 30% roughage throughout the experiment.

Blood Sampling for Progesterone

Blood samples were collected from each cow by jugular venepuncture at 3-d intervals from the start of the experiment to the start of the breeding season. Serum was assayed for progesterone concentration using a solid-phase RIA kits (Diagnostic Products Corp., Los Angeles, CA) validated for bovine serum in our laboratory (Custer et al., 1990). Intra- and interassay CV for a serum pool that contained 2.55 ng/mL were 0.4 and 7.4%, respectively; and for a pool that contained 7.49 ng/mL were 11 and 3.4%, respectively. Progesterone concentrations patterns were used to assess the resumption of ovarian cycling activity. A rise in progesterone concentration of > 0.5 ng/ml in three consecutive that was maintained for one or two additional samples provided evidence that cows resumed ovarian cycling activity during the experimental period. The graphic representation of the pattern of progesterone concentrations used to validate this criterion is given in Fernandez et al. (1993) for first-calf suckled beef cows.

Estrous Synchronization, AI, and Pregnancy Diagnosis

Each cow was given exogenous progesterone via a controlled intravaginal drug release (CIDR) device starting on May 18 (d -10). Seven d later (d -3) CUDD and CIDR were removed and cows were given PGF_{2α} (25 mg/hd) intramuscularly. Cows were visually observed for estrus by thrice daily (0730, 1200, 1800 h) after PGF_{2α} injection. Cows that showed estrus within 60 h after PGF_{2α} injection were bred by AI 12 h later. Cows that did not show estrus by 60 h were given GnRH (100 µg/hd) intramuscularly, and bred by AI 72 h after CIDR removal (d 0). Artificial insemination was accomplished using semen from a proven bull and a single AI technician. Pregnancy was determined by transrectal ultrasonography of the uterine contents of each cow 35 d after timed AI.

Statistical Analyses

Calving date, cow BW, calf birth weight, calf sex ratio, dystocia score, and BCS were analyzed by separate ANOVA for a completely randomized design using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). The model included treatment. Means were separated by the PDIFF procedure of SAS. Intervals from the start of treatment to resumption of ovarian cycling activity were calculated by

assigning the cows the d of the lowest inflection point before the increase of greater than 0.5 ng/mL of progesterone in 3 consecutive samples. Cows not showing a rise in progesterone over three consecutive blood samples were assigned an interval from the start of treatment to PGF_{2α} injection. Interval from the start of treatment to resumption of ovarian cycling activity was analyzed by ANOVA for a completely randomized design using PROC GLM of SAS. The model included treatment. Proportion of cows cycling at the start of estrous synchronization, proportion of cows that exhibited estrus after PGF_{2α} injection, and AI pregnancy rates were analyzed by PROC FREQ of SAS.

Results

Calving date, cow body weight, calf birth weight, calf sex ratio, dystocia score, and body condition score did not differ between cows exposed to bull urine (BUE) and cows exposed to steer urine (SUE).

Cow BW and BCS change from the start of treatment until after ES did not differ ($P = 0.22$, and 0.98 , respectively) between BUE and SUE cows. There was no difference ($P = 0.17$) in the intervals from treatment to the resumption of ovarian cycling activity between BUE and SUE cows. Similarly, there was no difference in the interval from the start of treatment to resumption of ovarian cycling activity between BUE or SUE cows for cows that resumed or did not resume ovarian cycling activity before PGF_{2α} injection ($P = 0.40$ and $P = 0.75$ respectively).

Table 1. Number of animals per treatment and percentage of cows exhibiting estrus by 60 h after PGF_{2α}, and TAI and overall AI pregnancy rates for first-calf suckled beef cows exposed to mature bull urine (BUE) or exposed to steer (SUE) before the start of the estrous synchronization protocol

Item	Treatment		X^2	<i>P</i> value
	BUE	SUE		
n	19	19		
% cycling by the end of the exposure period	15.8% ^b	30.6% ^b	1.3	0.25
% in estrus by 60 h after PGF _{2α}	80.0% ^b	52.6% ^b	2.9	0.09
% of cycling cows in estrus by 60 h after PGF _{2α}	66.7% ^b	33.3% ^b	0.9	0.34
% of anestrous cows in estrus by 60 h after	81.3% ^b	61.5% ^b	1.4	0.24
TAI pregnancy rate	100% ^b	66.7% ^b	1.7	0.19
AI pregnancy rates ^a	89.5% ^b	57.9% ^c	4.9	< 0.05

^aAI pregnancy rates equal cows bred 12 h after estrus and cows bred at TAI at 35 d after TAI.

^{b,c}Percentages within in rows that lack a common superscript differ.

There was no difference ($P = 0.25$) in the proportion of cows cycling by the end of the exposure period between BUE and SUE cows (Table 1).

Proportions of cycling and anestrus cows that exhibited estrus by 60 h after PGF_{2α} did not differ ($P = 0.34$ and 0.24 , respectively) between cows BUE and SUE cows (Table 1). Therefore, data were pooled for cycling and anestrus cows that exhibited estrus by 60 h after PGF_{2α} for cows BUE and SUE cows. The proportion of cows that exhibited estrus by 60 h after PGF_{2α} did not differ ($P = 0.09$; Table 1) between BUE and SUE cows.

Timed AI pregnancy rates did not differ ($P = 0.19$) BUE and SUE cows (Table 1). Therefore, data for cows bred by TAI and cows bred 12 h after estrus were pooled within treatments for AI pregnancy rates. Overall AI pregnancy rate was greater ($P < 0.05$) for BUE cows than for SUE cows (Table 1).

Discussion

The physical presence of bulls decreases the postpartum anestrus interval in first-calf suckled beef cows (Custer et al., 1990; Fernandez et al., 1993; 1996). Cows exposed to the excretory products of bulls resumed ovarian cycling activity earlier after calving than cows not exposed to bulls (Joshi et al., 2002). From these data it appears that bulls excrete a pheromone into the urine, feces, or from cutaneous glands that may initiate a neuroendocrine-endocrine cascade which results in the resumption of ovarian cycling activity. Most male to female interactions that alter the reproductive activity of the female are mediated by pheromones excreted in the urine of males (for review, see, Vandenberg, 1983). Thus, the most likely excretory product to evaluate for pheromonal activity in the biostimulatory effect of bulls is urine.

The objectives of this experiment were to determine if bull urine exposure alters the resumption of ovarian cycling activity before the breeding season and breeding performance of first-calf beef cows. We found that exposing cows to mature bull urine had no effect on the interval to resumption of ovarian cycling activity and did not increase the proportion of cows that started to cycle before the breeding season.

The percentage of SUE cows cycling by the end of the exposure period was comparable to the percentage of cows cycling that were not exposed to bulls in previous years (Berardinelli et al., 2004; Joshi et al., 2002). Only 15% of BUE cows were cycling by the end of the exposure period. This number is quite low considering cows were 92 to 111 days postpartum by the end of the exposure period. These data are contrary to those of Joshi et al. (2002) who reported that more cows exposed to the excretory products of bulls resumed cycling activity than cows exposed to their own excretory products or cows not exposed to bulls or their own excretory products. These results indicate that continuous exposure of postpartum anestrus cows to mature bull urine does not alter the occurrence of resumption of ovarian cycling activity.

In regard to breeding performance, 14 out of 19 BUE cows and only 10 of 19 SUE cows exhibited estrus within 60 h after PGF_{2α}. Although this difference is not statistically significant it is not consistent with previous experiments that found no indication for an improvement in response to estrous synchronization between cows exposed to bulls or cows not exposed to bulls (Anderson et

al., 2002; Joshi et al., 2002). Overall AI pregnancy rate was 31% higher for BUE than SUE. This result is not consistent with that of Anderson et al. (2002) who reported that AI pregnancy rates did not differ among; cows exposed to the excretory products of bulls, cows exposed to their own excretory products, cows exposed to the physical presence of bulls, and cows not exposed to bulls or their own excretory products. One difference between Anderson et al. (2002) and the present experiment is the use of CIDR. Progesterin was not used by Anderson et al. (2002) and recently, Stevenson et al. (2003) reported that progesterin treatment concurrent with a GnRH-based ES protocol improved pregnancy rates in suckled beef cows after AI. Thus, it appears that bull urine exposure in conjunction with an estrous synchronization protocol that included progesterin (CIDR), PGF_{2α}, GnRH and timed AI improved breeding performance in first-calf beef cows.

In conclusion, continuous exposure of first-calf suckled beef cows to bull urine did not alter the interval from treatment to the resumption of ovarian cycling activity or proportion of cows cycling before the beginning of the breeding season. However, continuous exposure to mature bull urine before an estrous synchronization protocol that included progesterin (CIDR), PGF_{2α}, and GnRH increased AI pregnancy rate. Therefore, it is possible that bull urine may contain a pheromone(s); continuous exposure to this pheromone(s) did not induce the resumption of ovarian cycling activity. However, continuous exposure to this pheromone(s) before the breeding season may improve the breeding performance of first-calf suckled beef cows.

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