

# Resumption of postpartum luteal function of primiparous, suckled beef cows exposed continuously to bull urine<sup>1,2</sup>

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**ABSTRACT:** The objective of this experiment was to determine if continuous exposure to bull urine alters resumption of ovarian cycling activity of primiparous, suckled beef cows. We tested the hypotheses that interval from urine exposure to resumption of luteal activity and proportions of cows that resume luteal activity by the end of the urine-exposure period do not differ between cows exposed to mature bull urine or steer urine. Thirty-eight Angus (A) × Hereford (H) cows, 4 mature A × H bulls and four 10-mo-old A × H steers, were used in this study. Cows were stratified by calving date, cow BW, calf BW, calf sex, dystocia score, and BCS; fitted with a controlled urine delivery device 2 wk before the start of treatments; and assigned randomly to be ex-

posed continuously (24 h/d) to bull urine (n = 19) or steer urine (n = 19) beginning 40 d after calving. Urine was collected from bulls and steers every third day of the experiment. Blood samples were collected from cows starting on d 0 and every third day thereafter until the end of the exposure period (~64 d). Likewise, controlled urine delivery devices were filled and refilled on the same schedule. Neither interval from urine exposure to resumption of luteal activity nor proportions of cows that resumed luteal activity during the urine-exposure period differed between cows exposed to bull urine or steer urine. We concluded that continuous exposure to mature bull urine does not affect resumption of luteal activity of primiparous, suckled beef cows.

**Key words:** biostimulation, bovine, bull urine, pheromone, postpartum interval

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## INTRODUCTION

Postpartum, primiparous cows exposed to bulls have shorter anestrus intervals to resumption of ovulatory cycles than cows not exposed to bulls (Custer et al., 1990; Fernandez et al., 1993, 1996). The mechanism of the biostimulatory effect of bulls on postpartum cows is not well understood. However, exposing postpartum, anovular cows to excretory products of bulls for 12 h/d for 70 d mimicked the effect of the physical presence

of bulls in accelerating resumption of ovulatory cycles (Berardinelli and Joshi, 2005a). This indicates that the biostimulatory effect of bulls involves a pheromonal mechanism. In mammals, male signaling and priming pheromones are predominantly contained in and released from urine (for review, see Izard, 1983). Baruah and Kanchev (1993) reported that oronasal administration of bull urine to cows on d 7 after calving increased LH and FSH within 80 min, supporting the idea that bull urine may contain a pheromone that affects the reproductive neuroendocrine-endocrine system of postpartum anovular cows.

The objective of the present experiment was to determine if continuous exposure to bull urine before the breeding season accelerates resumption of ovarian luteal activity of primiparous, suckled beef cows. We tested the null hypotheses that interval from the start of urine-exposure to resumption of luteal activity and proportion of cows resuming luteal function before the breeding season do not differ between cows exposed continuously to mature bull or steer urine.

## MATERIALS AND METHODS

Animal care, handling, and protocols used in this experiment were approved by the Montana State Uni-

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### *Animals and Treatments*

Thirty-eight 2-yr-old Angus-Hereford primiparous suckled beef cows, 4 epididymectomized Angus-Hereford bulls, and four 10-mo-old Angus-Hereford steers were used in this experiment conducted at the Montana State University Livestock Teaching and Research Center, Bozeman.

Cows and calves had no contact with bulls or their excretory products since the previous breeding season. Average calving date was Feb. 9 (range: January 25 to February 24). Two weeks before urine exposure, 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). Cows were then assigned randomly within strata to bull urine exposure (BUE) or steer urine exposure (SUE) 39 to 42 d after calving. The average length of exposure was 64 d.

### *Animal Housing Areas*

Two lots were used for this experiment, designated north and south by their geographic location. Each lot contained 4 pens (41 × 18 m) that were similar in east-west configuration, bunk space, aspect, slope, and connection to open-shed shelters. Cows were allowed to move between 2 pens in each lot. Lots were approximately 0.35 km apart. These lots and arrangements have proven to be effective in previous experiments involving bull-cow interactions (Berardinelli and Joshi, 2005a,b). The south lot had not held bulls for more than 7 yr, and the north lot had not held bulls for 10 mo. Bulls and steers were housed in 2 separate pens approximately 80 m apart and in a separate lot area approximately 0.4 km north of the lots that housed cows.

### *Controlled Urine Delivery Device*

Continuous exposure to urine was accomplished by fitting a CUDD to the ventromedial portion of the neck between the larynx and 7.5 cm cranial to the sternum of each cow. A CUDD consisted of a neoprene bag, 30-cm long, 17.5-cm wide, with an inner pouch and outer nylon fenestrated barrier, and a closure-flap that spanned from the inner pouch to the outer fenestrated barrier. The bottom of the bag was pleated, allowing for the expansion and contraction of the bag contents. The inner pouch contained a human urinary leg bag (Cat. No. 9814, Hollister Incorporated, Libertyville, IL), which held 750 mL of fluid and served as a reservoir for urine. The outer, fenestrated barrier contained a chemical-resistant cellulose sponge, 17 × 10.8 × 4.5 cm (length × width × depth).

A lumen was made along the entire length of the middle of the sponge to accommodate a urine flow-control valve. Flow-control valves were approximately 12.5

cm long and 1.25 cm in diameter and made of polypropylene tubing. Each valve was fitted into the lumen of each sponge and connected to the reservoir by a 0.953 × 0.635-cm, elbow-shaped, nylon hose-barb fitting. Elbows were placed through the neoprene dividing the inner and outer pouches on the ventral end of the bag. The barbed end of an elbow was pressure fitted into a reservoir, and the threaded end was connected to the flow-control valve using a small hose clamp. A rubber stopper (#00) capped the end of each valve distal to the elbow. Urine flowed from the valve and soaked the sponge through an outlet hole (23 ga.) approximately 2 cm from the capped end.

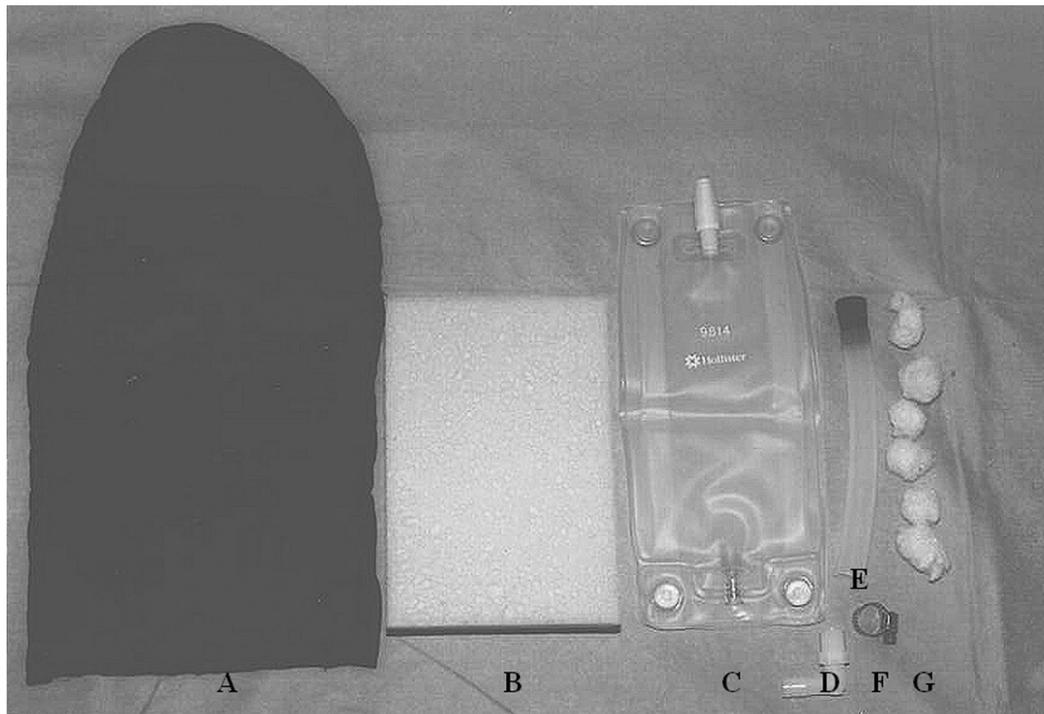
The lumen of each valve was filled with 3 g of polyester fiber packed tightly to 5 cm below the outlet hole and loosely packed to 2.5 cm above the elbow. Polyester fiber served to regulate urine flow-rate from the reservoir to the sponge for approximately 3 d.

To summarize, urine flowed from the reservoir through the elbow into the valve out of the 23 ga. outlet hole and soaked the sponge in the outer fenestrated barrier of the CUDD. Figure 1 illustrates components used in the construction of a CUDD. Laboratory tests, using distilled water as the medium, confirmed that fluid flowed from the reservoir and into the sponge, keeping the sponge moist for a period of 3 d.

### *CUDD Attachment*

Two weeks before the start of treatment, each cow was fitted with a 7.5-cm diam. ring placed through the loose skin in the midline of the dewlap, 7.5 cm cranial to the sternum. The ring was plastic-coated steel cable, 5 mm in diam. The loose skin and tissue next to the brisket were shaved, and 5 to 7 mL of lidocaine hydrochloride was injected s.c. to desensitize the area for cable insertion. A 1-cm-long incision was made through the skin and a trochar fitted into the lumen of a 9-mm steel cannula was inserted into the incision, through the soft tissue, and out the opposite side of the dewlap. The trochar was removed from the cannula, and the cable was then inserted through the lumen of the cannula, and the cannula was removed from the dewlap, leaving the piece of cable extended on each side of the dewlap.

The cable ends were then crimped together to form a ring. This ring served as a point of attachment for the ventral mount of each CUDD. At the start of treatment, CUDD were attached to each cow using an elastic band (5 cm wide) attached to the dorsal end of the CUDD near the flap and tied around the neck of the cow; the ventral end of the CUDD was attached to the ring, using 7.5-cm-long plastic zip-ties. The elastic strap allowed the CUDD to move laterally on each side of a cow's neck, with the bottom of the bag firmly attached to dewlap ring, keeping the bag parallel to the cranial aspect of the neck and sternum.



**Figure 1.** Components of controlled urine delivery device (CUDD). Objects in the picture are listed from left to right: A) neoprene bag with inner and outer pouch, B) cellulose sponge, C) urine reservoir, D) polyethylene elbow, E) tube for flow control valve with top rubber plug and 23-ga. hole, F) small hose clamp, and G) polyester fiber placed inside flow control valve.

### *Urine Collection and Handling*

Bull and steer urine was collected 1 d before the start of treatment and every 3 d thereafter until the end of the exposure period. Urine was collected from bulls and steers individually using a urine collection facility that consisted of 4 stalls raised 26 cm above a cement floor. Each stall was gated and measured 1 m wide  $\times$  1.5 m long  $\times$  1.15 m high. The stall floors consisted of 2 planks 25 cm wide and 5 cm thick placed at the front and back of the stall to support the weight of the bull or steer. Plastic-coated steel grating, 1.25 cm thick, was placed on top of, and secured to, the planks. This arrangement allowed for support of the weight of the animal and insured that urine flowed from the prepuce over an inert surface into a collection pan.

The collection pans were made of polyethylene and were 75 cm long, 62 cm wide, and varied in depth from 2 cm in the back to 6 cm in the front. Each urine collection pan was placed directly beneath the plastic grate and held in place by 2 pieces of angle iron attached to the underside of each stall. A 2.75-cm hole was drilled at the lowest point in the pan. A polyethylene connector, 2.5 cm in diam., was glued inside the hole. A faucet screen was then placed into the lumen of the connector to hinder the flow of debris through the connector. Attached to the connector was a piece of latex tubing 1.5 m long. This tubing was connected to a 2-L collection bag similar to that used for i.v. administration of fluids

to human patients. A separate set of pans was used for collecting urine from bulls and steers. The collection pans, tubing, and collection bags were arranged in such a way that insertion and extraction of pans and changing of tubing and collection bags was time and labor efficient and did not place undue stress on the bulls or steers.

Urine flowed from the prepuce of the bull or steer, through the plastic, grated floor into the urine collection pan, screen, connector, and latex tubing into a collection bag. When the collection bags were filled with urine they were replaced with empty collection bags. Each full collection bag was then placed on ice and stored in a cold room at 4°C until the next day, when CUDD were filled and refilled. Bags and tubing for bull urine and steer urine were stored in 2 cold rooms to prevent cross-contamination.

Urine collections from steers and bulls were performed on different days 24 h apart. The collection facility was washed with soap and water between collection periods. Collection periods varied from 4 to 8 h on any given day for bulls and steers, depending on the quantity of urine needed to fill the CUDD reservoirs and the urinary output of the bulls and steers.

### *CUDD Filling*

Urine collected the previous day was pooled and transferred into clean i.v. collection bags. These bags

were suspended 2 m above the head of the cows. A 2.5-m length of latex tubing was fitted to the effluent opening of the collection bag and secured with a plastic hose clamp. Each cow was then restrained in a standard chute, head-catch system. After the cow was in position, the CUDD closure-flap was opened, opening the urine reservoir. The latex tubing was then attached to the CUDD-effluent opening of the urine reservoir. Urine flowed from the collection bag through the latex tubing and into the reservoir of the CUDD (~750 mL of urine). After the reservoir was filled, the CUDD flap was closed and the filling procedure was complete.

Collection and transfer bags and all latex tubing were emptied of any remaining urine and rinsed thoroughly 3 times in hot tap water followed by 2 rinses in distilled water. Bags and tubing for bull urine and steer urine were washed, rinsed, dried, and stored in separate washing and drying rooms to prevent cross-contamination. This procedure was repeated every 3 d throughout the experiment. At the time of filling, each CUDD was inspected for nonfunctioning parts and was repaired as necessary.

### 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. After cows and calves were moved into the pens, they were given free access to the same hay, 0.5 kg·head<sup>-1</sup>·d<sup>-1</sup> of 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 (50% corn and 50% barley) and 30% roughage (ground grass-alfalfa hay) throughout the experiment.

### Blood Sampling

Blood samples were collected from each cow by jugular venipuncture at 3-d intervals during the exposure period (~64 d). Serum was analyzed for progesterone concentration in duplicate using 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 2.6 ng/mL of progesterone were 0.4 and 7.4%, respectively; and 3.4 and 11.0%, respectively, for a pool that contained 7.5 ng/mL of progesterone. Progesterone concentration patterns were used to determine the occurrence of resumption of luteal activity and the intervals from the start of urine exposure to resumption of luteal cycling activity.

A baseline increase of progesterone concentration that exceeded 1 ng/mL in 3 consecutive samples was

**Table 1.** Number of cows per treatment and least squares means for calving date, cow BW at the start of treatment, cow BW change, calf BW at the start of treatment, BCS, BCS change, calf sex ratio, dystocia score, and interval from exposure to resumption of luteal activity for primiparous, suckled beef cows exposed to bull urine (BUE) or exposed to steer urine (SUE)

Item	Treatment		SEM	P-value
	BUE	SUE		
No. of cows	19	19		
Calving date <sup>1</sup>	39	39	9.22	0.98
Cow BW, kg	557	550	40.9	0.59
Cow BW change <sup>2</sup>	-37.2	-22.3	21.5	0.22
Calf BW, kg	35	39	9.1	0.92
BCS	5.1	5.2	0.34	0.60
BCS change <sup>2</sup>	-0.04	-0.04	0.35	0.98
Calf sex ratio <sup>3</sup>	0.47	0.60	0.51	0.44
Dystocia score <sup>4</sup>	1.00	1.05	0.16	0.34
Interval, <sup>5</sup> d	62.5	55.8	14.6	0.17

<sup>1</sup>Day of year.

<sup>2</sup>Cow BW and BCS changes are differences from the start of treatment to the end of treatment.

<sup>3</sup>Calf sex ratio = ratio of male to female calves; 1 = male and 0 = female.

<sup>4</sup>Dystocia score: 0 = no assistance to 5 = Caesarean section.

<sup>5</sup>Days from the start of exposure to resumption of luteal activity.

used as the criterion to determine the occurrence of resumption of luteal activity. Intervals from the start of urine exposure to resumption of luteal activity were determined by the number of days from the start of urine exposure to the lowest inflection point before an increase in progesterone that exceeded 1 ng/mL in 3 consecutive samples. Cows that failed to exhibit an increase in progesterone over 3 consecutive blood samples were assigned an interval from the start of treatment to the end of treatment.

### 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, and means were separated by the PDIF procedure of SAS. Intervals from the start of urine exposure to the resumption of luteal activity were analyzed by ANOVA for a completely randomized design using PROC GLM of SAS. Proportions of cows that resumed luteal activity by the end of the exposure period were analyzed by  $\chi^2$  analyses using the PROC FREQ procedure of SAS.

## RESULTS

Calving date, cow body weight, calf birth weight, calf sex ratio, dystocia score, and BCS did not differ between BUE and SUE cows (Table 1). Similarly, cow BW and BCS change from the start of the exposure period until

the end of the exposure period did not differ between BUE and SUE cows (Table 1).

There was no difference in the intervals from the start of the exposure period to the resumption of luteal activity between BUE and SUE cows (Table 1). Likewise, proportions of cows that resumed luteal activity by the end of the exposure period did not differ between BUE (15%) and SUE cows (33%).

## DISCUSSION

This study was designed specifically to test whether bull urine contains a pheromone that is involved with the biostimulatory effect of bulls for accelerating resumption of luteal activity in postpartum, suckled cows. It was based on the findings of Berardinelli and Joshi, (2005b) that exposing cows to the excretory products of bulls for 12 h daily had the same biostimulatory effect as the physical presence of bulls. Also, primer pheromones of males that affect reproductive function of conspecific females are excreted into the environment with urine (for review, see Izard, 1983). Cows treated with androgens, or gonadal steroids, can elicit the same biostimulatory effect as bulls (Burns and Spitzer, 1992; Berardinelli and Joshi, 2005a,b). Therefore, to control for the possible negative or positive effect of urine exposure on the resumption of ovarian cycling activity in the current study, bovine urine devoid of gonadal steroid influence (i.e., steer urine) was used. The results of the present experiment indicate that exposing cows to mature bull urine 24 h daily, with this delivery system, had no effect on the interval to resumption of luteal activity and did not increase the proportion of cows that initiated estrous cycles before the end of the exposure period. There are 3 possible explanations for these results. First, the delivery system we employed somehow failed to deliver the pheromone into the environment. This seems unlikely, because in the current study observations every third day throughout the experiment indicated an ample amount of urine was flowing through the CUDD and into the environment. The second explanation may be that urine is not the media by which bulls deliver pheromonal cues to cause a biostimulatory effect. The "male effect" in sheep and goats appears to be mediated by factors associated with male fleece and urine (Gelez and Fabre-Nys, 2004). Bovine oronasal administration of bull urine accelerated age at puberty in heifers (Izard and Vandenberg, 1982). Baruah and Kanchev (1993) reported that oronasal administration of bull urine increased serum LH and FSH concentrations within 80 min after administration on d 7 after calving in dairy cows. Thus, in cattle it appears as though bull urine is the likely source of pheromones that mediate the biostimulatory effect of bulls on resumption of luteal activity in postpartum anestrous cows.

The third and most likely explanation is that continuous (24 h/d) pheromonal stimulation was an inappropriate mode of stimulation to cause resumption of luteal

activity. The manner by which the biostimulatory stimulus is presented to the cow might explain the results observed in this study. Bulls do not cause a biostimulatory effect if they are intermittently (2 h every third day) exposed to cows (Fernandez et al., 1996); however, bulls do have a biostimulatory effect if cows are exposed to the continuous physical presence of bulls (Custer et al., 1990) or the excretory products of bulls 12 h daily (Berardinelli and Joshi, 2005b). These results imply there is some minimum quantity of bull exposure needed to cause a biostimulatory effect. We assumed that this quantity was met or exceeded in the current study by exposing cows to bull urine continuously 24 h daily (i.e., if a little is good, more is better). Presenting the stimulus in this manner did not produce the expected result. Considering the natural interactions between bulls and cows, cows in pens or pastures come into contact with bulls periodically and repeatedly throughout the day and cows might be exposed to pheromonal stimulation periodically through a day and repeatedly over a course of days. In the present experiment cows were continuously (24 h/day) exposed to bull urine, which may not mimic natural bull-cow interactions. These observations indicate it is entirely possible that cows in this experiment were overstimulated by continuous bull urine exposure, indicating the necessity of a period of nonstimulation following periods of hyperstimulation for the biostimulatory effect of bulls.

In conclusion, bull urine exposure of primiparous suckled beef cows did not decrease the interval from calving to the resumption of luteal activity or increase the proportion of cows initiating estrous cycles before the beginning of the breeding season. These results do not preclude the possibility that bull urine contains a pheromone(s) but indicate the mode of pheromonal stimulation may be an important factor in the biostimulatory effect of bulls on resumption of luteal activity in postpartum, suckled beef cows.

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