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Putative urinary pheromone of bulls involved with breeding performance of primiparous beef cows in a progestin-based estrous synchronization protocol[1]

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ABSTRACT: The objective of this study was to determine if factors associated with the biostimulatory effect of bulls alter breeding performance of primiparous, suckled beef cows using a progestin-based estrous synchronization protocol. We tested the hypotheses that the estrous synchronization response and AI pregnancy rates differ among cows exposed to bulls, continuously exposed to bull urine, and exposed to fence-line contact with bulls or cows not exposed to bulls or bull urine. Data were collected from 3 experiments performed over consecutive years. Cows were assigned to the following treatments: bull exposure (BE; n = 26) or no bull exposure (NB; n = 25) in Exp. 1, bull urine exposure (BUE; n = 19) or steer urine exposure (SUE; n = 19) in Exp. 2, and fence-line contact with bulls (BFL; n = 26) or no bull exposure (NB; n = 26) in Exp. 3. Synchronization protocols in each experiment included the use of a controlled internal drug release device (d –10), $PGF_{2\alpha}$ (d –3), and GnRH and fixed-time AI (TAI; d 0). Cows that were observed in estrus by 60 h after $PGF_{2\alpha}$ were inseminated 12 h later. Cows not observed in estrus by 60 h after $PGF_{2\alpha}$ were TAI at 72 h and given GnRH (100 μg). Pregnancy was determined by ultrasonography 35 d after TAI. In Exp. 1, 2, and 3, cows were exposed directly to bulls, bull urine, or bull fence-line contact for 35, 64, and 42 d, respectively. Data were analyzed between treatments within each experiment. The proportion of estrous cycling cows did not differ between treatments at the beginning of each experiment; however, more ($P < 0.05$) BE and BFL cows were estrous cycling at the beginning of the estrous synchronization protocol than NB cows in Exp. 1 and 3. The proportion of cows that showed estrus and interval to estrus after $PGF_{2\alpha}$ did not differ between treatments in Exp. 1 and 3. However, in Exp. 2, more BUE cows tended ($P = 0.09$) to have shorter intervals to estrus and to exhibit estrus after $PGF_{2\alpha}$ than SUE cows. Overall, AI pregnancy rates were greater ($P < 0.05$) for BE and BUE cows than for NB and SUE cows in Exp. 1 and 2, respectively. There was no difference in AI pregnancy rates between BFL and NB cows in Exp. 3. The presence of bulls and exposure to bull urine appeared to improve breeding performance of primiparous beef cows using a progestin-based estrous synchronization protocol, whereas fence-line bull exposure was insufficient to cause this biostimulatory effect. We propose that a novel urinary pheromone of bulls may be responsible for the enhancement of fertility in the primiparous, postpartum cow.

Key words: biostimulation, bovine, breeding performance, controlled internal drug release device, pheromone, postpartum

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INTRODUCTION

Prolonged postpartum anestrus in primiparous cows is a major cause of cows failing to rebreed or breeding late in the breeding season (Short et al., 1994). For this reason, it is a challenge to successfully synchronize estrus in primiparous, suckled beef cows. Primiparous, suckled cows exposed to bulls or bull excretory products after calving resume luteal function sooner than cows not exposed to bulls or excretory products of bulls (Custer et al., 1990; Berardinelli and Joshi, 2005b).

Berardinelli et al. (2007) reported that fixed-timed AI pregnancy rate and overall AI pregnancy rate of primiparous cows were improved by exposing the cows

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to bulls before, during, and after a GnRH-based estrous synchronization protocol. The questions asked were as follows: 1) can the biostimulatory effect of bulls be used to improve the estrous synchronization response and AI pregnancy rates of primiparous, suckled beef cows using a progestin-based estrous synchronization protocol, and, if so, 2) is this biostimulatory effect of bulls mediated by a pheromone(s) in bull urine?

The objectives of the current study were to determine if short-term exposure to a bull, continuous exposure to bull urine, or fence-line exposure to a bull alters the estrous synchronization response (interval to estrus after $PGF_{2\alpha}$ and proportion that exhibit estrus) and AI pregnancy rates of primiparous, suckled beef cows when using a progestin-based protocol that includes a controlled internal drug release device (**CIDR**), $PGF_{2\alpha}$, and fixed-time AI (**TAI**) with concurrent administration of GnRH 72 h after $PGF_{2\alpha}$.

MATERIALS AND METHODS

Animals and Treatments

Animal care, handling, and protocols used in these experiments were approved by the Montana State University Institutional Large Animal Care and Use Committee. The experiments were conducted over 3 consecutive years at the Montana State University Livestock Teaching and Research Center, Bozeman.

In each experiment, the cows were maintained in a single pasture after calving and had not been exposed to bulls or bull excretory products since the previous breeding season. Before the beginning of treatment, the cows were stratified by calving date, calf BW, cow BW, cow BCS, and dystocia score and assigned randomly within each experiment to 1 of 2 treatments: physical presence of bulls (BE) or no bull exposure (NB) in Exp. 1, continuous bull urine exposure (BUE) or continuous steer urine exposure (SUE) in Exp. 2, and fence-line exposure to bulls (BFL) or no bull exposure (NB) in Exp. 3. Thus, we tested the following hypotheses: 1) the proportions of primiparous, postpartum cows that resume luteal function before the breeding season, 2) the estrous synchronization response, and 3) the AI pregnancy rates differed among BE or NB cows in Exp. 1, BUE or SUE cows in Exp. 2, and BFL or NB cows in Exp. 3.

The calving season for cows in each of the experiments began January 27 and ended on March 10. Average calving dates were February 16, 9, and 10 for cows in Exp. 1, 2, and 3, respectfully. Treatment began 28 and 35 d before the beginning of the estrous synchronization protocol in Exp. 1 and 3, respectfully. In Exp. 2, cows were exposed to urine beginning 39 to 42 d after calving. In Exp. 1, 2, and 3, cows were exposed directly to bulls, bull urine, or bull fence-line contact for 35, 64, or 42 d, respectively. Cows and calves remained in their respective treatments throughout each experiment until TAI (d 0).

Animal Housing Areas and Bull Exposure

The same 2 areas were used each experiment, designated as the north and south lots 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-front 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 (Fernandez et al., 1993; Berardinelli and Joshi, 2005a,b).

In Exp. 1, bulls were housed in the north lot and were allowed to move freely within the pens that housed BE cows, whereas NB cows were housed in the south lot. In Exp. 2, BUE cows were housed in the north area lot, and SUE cows were housed in the south lot; bulls and steers were housed in 2 separate pens approximately 80 m apart and in a separate lot approximately 0.4 km north of the lots that housed the cows. In Exp. 2, urine was collected from bulls and steers, and cows were continuously exposed to urine by using a controlled urinary delivery device fitted to each cow, as described by Tauck et al. (2006). In Exp. 3, bulls were housed in small enclosures within the 2 pens that housed BFL cows in the north lot, and a vacant enclosure (which housed no animals) that equaled the size of the enclosure that housed the bulls in the north area lot was built within the pens that housed NB cows in the south lot. A detailed description of the experimental setup of the pen areas used in Exp. 3 is given in Berardinelli and Tauck (2007). Under these conditions, pen and treatment may be confounded; however, no effect of pen or pasture has been observed in similarly designed studies in these facilities (Custer et al., 1990; Fernandez et al., 1996; Tauck et al., 2006) or in studies that have been designed to evaluate specifically the effect of pen and the biostimulatory effect of bulls (Landaeta-Hernandez et al., 2004, 2006).

Nutrition

In each experiment, cows had free access to good-quality chopped, mixed-grass alfalfa hay and any pasture grasses that were available before the beginning of each experiment. After cows and calves were moved into the pens, they were given free access to the same hay, cracked barley (0.5 kg/cow per d), 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).

In Exp. 1, bulls were housed with cows at the beginning of treatment and were fed the same diet as the cows. In Exp. 2, bulls had ad libitum access to chopped barley hay. During urine collection periods in Exp. 2, bulls were fed 0.5 kg of cracked barley and good-quality chopped, mixed-grass alfalfa hay. Steers in Exp. 2 were fed a finishing ration that consisted of 70% concentrate

(50% corn and 50% barley) and 30% roughage (ground grass and alfalfa hay), on an as-fed basis, throughout the experiment and during the urine collection periods. In Exp. 3, bulls within the small enclosure were fed ad libitum mixed grass-alfalfa hay. Animals in each experiment had free access to water and a mineral supplement.

Estrous Synchronization, AI, and Pregnancy Diagnosis

In each experiment, cows were given exogenous progesterone via a CIDR 10 d TAI on d 0. After 7 d, CIDR were removed, and each cow received $PGF_{2\alpha}$ (25 mg/animal; ProstaMate dinoprost tromethamine, Phoenix Scientific Inc., St. Joseph, MO). Bull exposure, bull and steer urine exposure, and bull fence-line exposure ended in Exp. 1, 2, and 3, respectfully, 3 d after $PGF_{2\alpha}$. Cows were observed visually for estrus for 72 h after receiving $PGF_{2\alpha}$ from 0600 to 2400. Cows that showed estrus within 60 h after $PGF_{2\alpha}$ were inseminated by AI 12 h later. Cows that did not show estrus within 60 h were given GnRH (100 μg) and inseminated by AI 72 h after $PGF_{2\alpha}$ (i.e., TAI), at which time the treatments ended and cows were combined and managed as 1 group in each experiment. Cows were exposed to bulls 18 d later for 21 d after each experiment. Pregnancy was determined by transrectal ultrasonography of the uterine contents of each cow 35 d after TAI in each experiment.

Criteria for Resumption of Luteal Activity

Exp. 1 and 2. Blood samples were obtained from each cow in each treatment by jugular venipuncture every third day over the course of the exposure periods used in each experiment. Serum was harvested and stored at –20°C until assayed for progesterone. Progesterone was assayed using solid-phase RIA kits (Diagnostic Products Corp., Los Angeles, CA) validated in our laboratory for bovine serum (Custer et al., 1990). Intra- and interassay CV for serum pools that contained 0.42 ng/mL and 3.1 ng/mL were <10%, respectively. Changes in progesterone concentrations were used to assess the resumption of ovarian luteal function. An increase in baseline progesterone concentrations that exceeded 1.0 ng/mL in 3 consecutive samples was used as evidence of resumption of luteal activity in these experiments.

Exp. 3. Ovaries of each cow were examined by transrectal ultrasonography using an Aloka 200 ultrasongraph equipped with a 5-MHz rectal transducer (Aloka America, Wallingford, CT) at the beginning of the experiment and at 7-d intervals over the 42-d exposure period used in this trial. The presence of a corpus luteum in the same anatomical position of an ovary in 2 successive ultrasonograms was used as evidence of resumption of luteal activity.

Statistical Analyses

Data were analyzed between treatments within each experiment. Intervals from $PGF_{2\alpha}$ administration to estrus were analyzed by ANOVA for a completely randomized design using PROC GLM (SAS Inst. Inc., Cary, NC). The model included treatment, and means were separated by the PDIFF option of SAS. The proportions of cows that resumed luteal function and showed estrus after $PGF_{2\alpha}$ and AI pregnancy rates were analyzed by χ^2 analysis using the FREQ procedure of SAS. Differences between treatments were considered significant at $P < 0.05$, and trends in data were considered at $P < 0.10$.

RESULTS

The proportion of cows that had resumed luteal function at the beginning of each experiment did not differ between treatments. In Exp. 1 and 3, more ($P < 0.05$) BE (100%) and BFL (85.7%) cows had initiated luteal function at the beginning of the estrous synchronization protocol than NB cows (70.4 and 72.8%, respectively). However, in Exp. 2, there was no difference in the proportion of cows that resumed luteal function between BUE (15%) and SUE (33%) cows at the beginning of the estrous synchronization protocol.

The proportion of cows that showed estrus and interval to estrus after $PGF_{2\alpha}$ did not differ between treatment in Exp. 1 and 3 (Table 1); however, in Exp. 2, BUE cows tended ($P = 0.09$) to have shorter intervals to estrus, and more BUE cows tended ($P = 0.09$) to exhibit estrus after $PGF_{2\alpha}$ than SUE cows (Table 1). Pregnancy rates for cows inseminated 12 h after estrus and overall AI pregnancy rates were greater ($P < 0.05$) for BE and BUE cows than NB and SUE cows in Exp. 1 and 2, respectively (Table 1), whereas TAI pregnancy rates did not differ between BE and NB cows or between BUE and SUE cows in Exp. 1 and 2 (Table 1). In Exp. 3, pregnancy rates for cows inseminated 12 h after estrus, TAI pregnancy rates, and overall AI pregnancy rates did not differ between BFL and NB cows (Table 1).

DISCUSSION

Cows exposed to the excretory products of bulls resumed luteal function earlier after calving than cows not exposed to bulls (Berardinelli and Joshi, 2005b). This result indicates that bulls excrete a pheromone(s) via urine, feces, or cutaneous glands that may initiate the neuroendocrine-endocrine cascade, which results in the resumption of luteal function. The nature of this signal is unknown; however, most male-to-female interactions that alter the reproductive activity of the female are mediated by pheromones excreted in the urine of males (Izard, 1983). Thus, the most likely excretory product to evaluate for pheromonal activity in the biostimulatory effect of bulls is urine. The results for the resumption of luteal function for postpartum, anovular

Table 1. Estrous synchronization response and AI pregnancy rates to an estrous synchronization protocol for postpartum primiparous cows exposed to bulls (BE) or not exposed to bulls (NB) in Exp. 1, exposed continuously to bull (BUE) or steer (SUE) urine in Exp. 2, and exposed to fence-line contact with bulls (BFL) or not exposed to bulls (NB) in Exp. 3[1]

	Exp. 1				Exp. 2				Exp. 3			
Item	BE	NB	χ^2	*P*-value	BUE	SUE	χ^2	*P*-value	BFL	NB	χ^2	*P*-value
No. of cows	26	25			19	19			26	26		
Interval to estrus after $PGF_{2\alpha}$,[2] h	65.8	67.8		0.67	54.4	63.1		0.09	65.8	67.6		0.67
Proportion showing estrus after $PGF_{2\alpha}$, %	42.3	36.0	0.2	0.64	80.0	52.6	2.9	0.09	61.5	50	0.7	0.40
Pregnancy rate for cows inseminated 12 h after estrus, %	100	66.7	4.3	<0.05	86.7	50.0	4.0	<0.05	56.3	77.0	1.4	0.24
Pregnancy rate for cows inseminated at 72 h after $PGF_{2\alpha}$, %	73.3	56.3	1.0	0.32	100	60.0	2.2	0.13	60.0	77.0	0.8	0.38
Overall AI pregnancy rate, %	84.6	60.0	3.9	<0.05	89.5	55.0	5.7	<0.05	57.7	77.0	2.1	0.14

[1]The estrous synchronization protocol included a controlled internal drug release device (CIDR; 7 d), $PGF_{2\alpha}$ at the time of CIDR removal, and timed AI and GnRH 72 h after $PGF_{2\alpha}$.
[2]Standard error of the mean for interval to estrus after $PGF_{2\alpha}$: Exp. 1 = 17.0; Exp. 2 = 16.2; and Exp. 3 = 15.0.

cows (Exp. 2 of the current study) were reported by Tauck et al. (2006). In Exp. 2, the percentage of SUE cows that initiated luteal function by the end of the exposure period (~33%) was similar to the percentages of cows that had initiated luteal function and were not exposed to bulls in previous years (Berardinelli and Joshi, 2005a,b). More importantly, only 15% of BUE cows had initiated luteal function by the end of the exposure period. These data are contrary to those of Berardinelli and Joshi (2005b), who reported that more cows exposed to the excretory products of bulls resumed luteal function than cows exposed to their own excretory products or cows not exposed to bulls or their own excretory products. These results led us to ask the question, "Is a urinary pheromone involved in the biostimulatory effect of bulls that accelerates resumption of ovulatory cycles in postpartum, anovulatory cows?" Tauck et al. (2006) suggested that the discrepancy in these results may be related to the manner by which cows were exposed to bull urine [i.e., continuous exposure (24 h/d for >60 d) of postpartum anestrous cows to mature bull urine may not provide the appropriate pheromonal signal to alter the occurrence of resumption of luteal activity]. However, it is clear that both close physical presence of bulls and close fence-line contact of cows with bulls pheromonally stimulate acceleration of resumption of luteal activity in postpartum, anovulatory cows. Furthermore, Berardinelli et al. (2007) hypothesized that the pheromonally mediated biostimulatory effect of bulls may not only affect resumption of ovulation in postpartum, anestrous cows but may also influence breeding performance by directly affecting the ovary or reproductive tract physiology to enhance AI conception rates in GnRH-responsive cows.

The primary objective of the current study was based on the results reported in the study by Berardinelli et al. (2007) in which overall AI pregnancy rates using an estrous synchronization protocol that included GnRH followed 7 d later by $PGF_{2\alpha}$ and TAI were significantly improved by exposing postpartum, suckled cows to the biostimulatory effect of bulls. We hypothesized that the estrous synchronization response and AI pregnancy rate using a progestin-based estrous synchronization protocol that included a CIDR followed 7 d later by CIDR removal and $PGF_{2\alpha}$ administration and TAI in cows exposed to the biostimulatory effect of bulls would be similar to that obtained in the previous study. We found that neither short-term close physical contact (Exp. 1) nor close fence-line contact (Exp. 3) altered the estrous response (interval to estrus and proportion exhibiting estrus by 72 h) of cows after $PGF_{2\alpha}$. This result was similar to that reported by Berardinelli et al. (2007) for cows synchronized with a GnRH-based $PGF_{2\alpha}$ protocol. However, in Exp. 2, there was a tendency for more BUE cows (80%) to exhibit estrus within 60 h after $PGF_{2\alpha}$ than SUE cows (52.6%). This was an unexpected result in that only 3 of 19 BUE cows had resumed luteal function before the beginning of the estrous synchronization protocol, and one would expect that with a few cows that had initiated luteal function and greater number of anovular cows in this treatment that the estrous response (estrus within 72 h after $PGF_{2\alpha}$) would also be less (Lucy et al., 2001). This result indicates the possibility of a biostimulatory effect of continuous bull urine exposure of the estrous response in primiparous cows synchronized with a progestin-based estrous synchronization protocol. Whether this is a direct effect of altering ovarian follicular development or an indirect effect mediated by the hypothalamic-hypophyseal-ovarian axis is not clear.

The possibilities of a biostimulatory urinary pheromone(s) seem apparent when the collective results of the AI pregnancy rates between treatments and among experiments are examined. In Exp. 3, there was no difference in AI pregnancy rates between cows exposed to close fence-line contact with bulls and cows not exposed to bulls. This result is consistent with that of

Fike et al. (1996), who reported that fence-line contact of cows with bulls had no effect on AI pregnancy rates. However, overall AI pregnancy rates were greater for cows exposed to close physical contact with bulls (BE cows) and cows exposed continuously to mature bulls urine (BUE cows) than cows not exposed to bulls (NB cows) and cows exposed continuously to steer urine (SUE cows) in Exp. 1 and 2, respectfully. The overall AI pregnancy rate for BE cows in Exp. 1 is in agreement with and supports that of Berardinelli et al. (2007), who reported that overall AI pregnancy rates were improved by exposing postpartum cows to the close physical presence of bulls or to the excretory products of bulls before and during an estrous synchronization protocol that included GnRH followed 7 d later by $PGF_{2\alpha}$ and TAI. Perhaps the most surprising result of the current study was that AI pregnancy rates of the cows exposed continuously to mature bull urine (BUE cows) were significantly greater than cows exposed continuously to steer urine (SUE cows) before and during the estrous synchronization protocol in Exp. 2. It is important to note that visual inspection of the data for overall AI pregnancy rates for cows exposed to the physical presence of bulls (Exp. 1) and cows exposed continuously to bull urine (Exp. 2) are almost equal (84.6 and 89.5%, respectively) and appear to be greater than those for all other treatments. To our knowledge, this is the first report of the observation that bull urine may contain a pheromone(s) that has a biostimulatory effect on breeding performance of postpartum anovular cows using a progestin-based estrous synchronization protocol.

This unusual and interesting finding indicates the possibility that the biostimulatory effect of bulls on the reproductive processes of postpartum, anovular cows is more complex than has been previously thought. Although in each experiment the number of cows per treatment was less than desired from a statistical power perspective, taken together, we believe there is compelling evidence in the data to indicate a novel and second pheromonal component associated with the biostimulatory effect of bulls, one that can in some manner influence fertility at least after a progestin synchronization period. The first component of this biostimulatory effect of bulls appears to temporally accelerate the reproductive neuroendocrine-endocrine cascade that culminates in the resumption of ovulation and luteal function in postpartum anovulatory cows. This is evident in the data reported in the current study from Exp. 1 and 3 and numerous other studies. The second component we hypothesize to be associated almost exclusively with aspects of fertility (i.e., improved breeding performance). In order for this component to manifest itself, cows must be in very close approximation with the urine of bulls. Without this type of physical constraint, the reproductive performance or fertility enhancement effect of bulls will not occur. This novel hypothesis is supported by the results of Exp. 3, in which fence-line contact of cows with bulls failed or the pheromonal stimulus was insufficient to improve breeding performance, whereas continuous close physical association (Exp. 1) and continuous exposure to bull urine (Exp. 2) provided sufficient stimulus to enhance breeding performance in postpartum, primiparous cows.

In conclusion, continuous exposure of postpartum, anovular cows to bull urine or to close physical contact with mature bulls, but not fence-line contact, appeared to improve AI pregnancy rates in a progestin-based estrous synchronization protocol that included a CIDR followed 7 d later by CIDR removal and $PGF_{2\alpha}$ administration and TAI and GnRH. Furthermore, the collective results of the experiments reported herein indicate the possibility of a second biostimulatory component of the bull effect that is mediated by a urinary pheromone(s). The nature of these biostimulatory putative pheromone(s) and the physiological mechanism by which these components interact with the reproductive system and reproductive regulatory systems of postpartum cows will require further investigation.

LITERATURE CITED

Berardinelli, J. G., and P. S. Joshi. 2005a. Introduction of bulls at different days postpartum on resumption of ovarian cycling activity in first-calf beef cows. J. Anim. Sci. 83:2106–2110.

Berardinelli, J. G., and P. S. Joshi. 2005b. Resumption of postpartum ovarian cycling activity in primiparous restricted suckled beef cows exposed to a bull or excretory products of bulls or cows. J. Anim. Sci. 83:2495–2500.

Berardinelli, J. G., P. S. Joshi, and S. A. Tauck. 2007. Conception rates to artificial insemination rates in primiparous, suckled cows exposed to the biostimulatory effect of bulls before and during a gonadotropin-releasing hormone-based estrus synchronization protocol. J. Anim. Sci. 85:848–852.

Berardinelli, J. G., and S. A. Tauck. 2007. Intensity of the biostimulatory effect of bulls on resumption of ovulatory activity in primiparous, suckled, beef cows. Anim. Reprod. Sci. 99:24–33.

Custer, E. E., J. G. Berardinelli, R. E. Short, M. Wehrman, and R. Adair. 1990. Postpartum interval to estrus and patterns of LH and progesterone in first-calf suckled beef cows exposed to mature bulls. J. Anim. Sci. 68:1370–1377.

Fernandez, D., J. G. Berardinelli, R. E. Short, and R. Adair. 1993. Temporal requirement for the presence of mature bulls to alter the postpartum period to resumption of ovarian cycling activity and reproductive performance in first-calf suckled beef cows. Theriogenology 39:411–419.

Fernandez, D. L., J. G. Berardinelli, R. E. Short, and R. Adair. 1996. Acute and chronic changes in LH secretion, and postpartum interval to estrus in first-calf suckled beef cows exposed continuously or intermittently to mature bulls. J. Anim. Sci. 74:1098–1100.

Fike, K. E., E. G. Bergfeld, A. S. Cupp, F. N. Kojima, V. Mariscal, T. S. Sanchez, M. E. Wehrman, and J. E. Kinder. 1996. Influence of fenceline bull exposure on duration of anoestrus and pregnancy rate in beef cows. Anim. Reprod. Sci. 41:161–167.

Izard, M. K. 1983. Pheromones and reproduction in domestic animals. Page 253 in Pheromones and Reproduction in Mammals. J. G. Vandenbergh, ed. Acad. Press, New York, NY.

Landaeta-Hernandez, A. J., P. Melendez, J. Bartolome, D. O. Rae, and L. F. Archbald. 2004. Effect of biostimulation on uterine involution, early ovarian activity and first postpartum estrous cycle in beef cows. Theriogenology 61:1521–1532.

Landaeta-Hernandez, A. J., P. Melendez, J. Bartolome, D. O. Rae, and L. F. Archbald. 2006. Effect of biostimulation on the expression of estrus in postpartum Angus cows. Theriogenology 66:710–716.

Lucy, M. C., H. J. Billings, W. R. Butler, L. R. Ehnis, M. J. Fields, D. J. Kesler, J. E. Kinder, R. C. Mattos, R. E. Short, W. W. Thatcher, R. P. Wettemann, J. V. Yelich, and H. D. Hafs. 2001. Efficacy of an intravaginal progesterone insert and an injection of $PGF_{2\alpha}$ for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. J. Anim. Sci. 79:982–995.

NRC. 1996. Page 227 in Nutrient Requirements of Beef Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Short, R. E., R. A. Bellows, R. B. Staigmiller, D. C. Adams, and J. G. Berardinelli. 1994. Effects of suckling on postpartum reproduction. Page 179 in Factors Affecting Calf Crop. M. J. Fields and R. S. Sand, ed. CRC Press, Boca Roton, FL.

Tauck, S. A., J. G. Berardinelli, T. W. Geary, and N. J. Johnson. 2006. Resumption of postpartum luteal function of primiparous, suckled beef cows exposed continuously to bull urine. J. Anim. Sci. 84:2708–2713.