

Estrus and Luteal Function in Suckled Beef Cows That Were Anestrous When Treated with an Intravaginal Device Containing Progesterone With or Without a Subsequent Injection of Estradiol Benzoate¹

K. E. Fike*, M. L. Day[†], E. K. Inskeep[‡], J. E. Kinder^{2,*},
P. E. Lewis[‡], R. E. Short[§], and H. D. Hafs[¶]

Departments of Animal Science, *University of Nebraska, Lincoln 68583-0908,
[†]Ohio State University, Columbus 43210, and [‡]West Virginia University, Morgantown 26506;
[§]USDA, ARS, Livestock and Range Research Laboratory, Miles City, MT 59301;
and [¶]Department of Animal Science, Rutgers University, New Brunswick, NJ 08903

ABSTRACT: The objectives in this study were to determine whether treatment with progesterone (P_4) via an intravaginal device would induce estrus and development of corpora lutea (CL) with typical life spans and whether treatment with estradiol benzoate (EB) following device removal would enhance the responses. At treatment initiation (d 0), suckled beef cows ($n = 362$) that were anestrous received one of the following: 1) an intravaginal device containing P_4 for 7 d plus an injection of 1 mg of EB 24 to 30 h after device removal ($P_4 + EB$), 2) an intravaginal device containing P_4 for 7 d (P_4), 3) a sham device for 7 d plus an injection of 1 mg of EB 24 to 30 h after device removal (EB), or 4) a sham device for 7 d (control). Progesterone treatment increased the proportion of

cows that formed CL with a typical lifespan ($P < .001$) and exhibited behavioral estrus ($P < .05$). Treatment with EB in combination with P_4 increased ($P < .001$) the proportion of cows that exhibited estrus. Treatment with P_4 alone had no effect, but the combination of P_4 and EB increased ($P < .01$) the proportion of cows that formed short-lived or typical lifespan CL by the end of the experiment compared with untreated cows. In summary, treatment with P_4 increased the incidence of estrus and the proportion of cows that formed CL with a typical lifespan. Treatment with P_4 resulted in resumption of luteal function in suckled beef cows that were anestrous, with most cows developing CL with a typical lifespan, whereas EB enhanced the expression of estrus.

Key Words: Anestrous, Estradiol, Progesterone, Beef Cows

J. Anim. Sci. 1997. 75:2009-2015

Introduction

Conception must occur by approximately 85 d after calving for cows to maintain yearly calving intervals. If postpartum anestrous persists at initiation of breeding seasons, time of conception may be delayed or cows may fail to conceive during the breeding season, and this increases culling rate in herds and decreases net income of producers (Bellows et al., 1979; Werth et al., 1991). Treatments that induce onset of estrous cycles in postpartum anestrous cows should, therefore, improve reproductive efficiency.

Treatment with progestins induces estrous cycles in some cattle that are anestrous (Miksch et al., 1978; Smith et al., 1987; Anderson et al., 1996). Progestin pretreatment alters uterine function after the first postpartum ovulation and yields normal duration of luteal function (Cooper et al., 1991). Administration of estradiol benzoate (EB) following progesterone (P_4) withdrawal has been reported to enhance the incidence of ovulation in postpartum cows (Ulberg and Lindley, 1960; Saiduddin et al., 1968; Brown et al., 1972).

The objectives of this experiment were to determine whether 1) treatment with P_4 via an intravaginal device induces estrus and formation of corpora lutea (CL) with typical lifespans, and 2) treatment with EB following P_4 removal improves rates of behavioral estrus and formation of CL with typical lifespans in suckled beef cows that are anestrous.

¹This research was supported in part by grants in aid from InterAg, Hamilton, New Zealand.

²Correspondence: A224 Animal Science.

Received August 12, 1996.

Accepted March 20, 1997.

Materials and Methods

Beef cows ($n = 362$) from 25 to 50 d postpartum that were anestrous and nursing calves were used in four locations (Nebraska, $n = 101$; Ohio, $n = 92$; Montana, $n = 97$; and West Virginia, $n = 72$), with each location serving as a replicate. On average, cows were in their third parity during the experiment. Breeds of the cows at each location were primarily as follows: Montana, crossbreeds containing Red Angus, Charolais, Tarentais, Hereford, Angus, and Simmental; Ohio, Simmental \times Angus; West Virginia, Angus; Nebraska, 1/4 Hereford, 1/4 Angus, 1/4 Pinzgauer, and 1/4 Red Poll. Within each replicate, cows were stratified by calving date and assigned to receive one of four treatments arranged in a 2×2 factorial. Beginning on d 0 (day of treatment initiation) cows were treated with one of the following: 1) an intravaginal device containing P_4 (Eazi-Breed™ CIDR®, InterAg, Hamilton, New Zealand) for 7 d plus an injection of 1 mg of EB (CIDROL®, InterAg) 24 to 30 h after P_4 removal ($P_4 + EB$); 2) an intravaginal device containing P_4 for 7 d (P_4); 3) a sham device for 7 d plus an injection of 1 mg of EB 24 to 30 h after device removal (EB); or 4) a sham device for 7 d (control). The intravaginal device contained 1.9 g of P_4 and was designed to release amounts of P_4 that result in physiological concentrations of this hormone in circulation as detected during the luteal phase of the estrous cycle in cattle (Macmillan et al., 1991).

Body condition scores, based on a 1-to-9 scoring system (1 = thin and 9 = fat), were assessed for each animal on the day of device insertion. Mean body condition scores (mean, SD) of cows within each location were as follows: Montana, 4.6, 1.0; Nebraska, 4.1, 1.0; Ohio, 4.7, .7; West Virginia, 5.0, .6. Numbers of cows fitting within the following ranges of body condition scores are as follows (body condition score, number of cows): 2.5 to 3.4, 41; 3.5 to 4.4, 116; 4.5 to 5.4, 155; 5.5 to 6.4, 38; and 6.5 to 9, 9.

Blood Collection and Radioimmunoassay. Blood samples were collected on d -7, 0, 8, 15, and 22 (d 0 = device insertion) via the jugular or tail vein. Blood samples were placed on ice immediately after collection, then stored at 4°C for 24 h until centrifugation. Serum was decanted and stored at -20°C until assayed for concentrations of P_4 . Progesterone assays performed at Montana (Bellows et al., 1991), West Virginia (Sheffel et al., 1982), Nebraska (Bergfeld et al., 1996), and Ohio (Anderson et al., 1996) had inter- and intraassay CV less than 20 and 9%, respectively.

Response to Treatment. Luteal function throughout the experiment was assessed by monitoring changes in serum P_4 . Cows that were anestrous were required for the experiment; therefore, the data were not included in analyses from 52 cows that had elevated concentrations of P_4 on d -7 and/or 0, which indicated that

ovulation had occurred before treatment initiation. Eight cows in the experiment lost devices before the end of treatment, and data for these cows also were not used in analyses. Based on the following criteria regarding P_4 concentrations in serum, data for the remaining cows were included in the corresponding response category: 1) serum P_4 increase of no more than .5 ng/mL on d 8, 15, or 22 indicated absence of luteal function (anestrous); 2) basal serum P_4 on d 0 and 8, followed by an increase of at least .5 ng/mL on d 15 and remaining elevated by at least .5 ng/mL on d 22 indicated formation of CL no later than 4 d after device removal, and the CL had a typical lifespan (typical lifespan CL); 3) basal serum P_4 on d 0 and 8, followed by an increase of at least .5 ng/mL on d 15, but then a decrease to basal concentrations of P_4 by d 22 indicated formation of CL no later than 4 d after device removal, and a "short" estrous cycle (short-lived CL); 4) basal serum P_4 on d 0 and 8, followed by an increase of no more than .5 ng/mL on d 15, but then an increase of more than .5 ng/mL on d 22 indicated formation of CL beyond 4 d after device removal (late CL); and 5) basal serum P_4 on d 0, followed by an increase of at least .5 ng/mL on d 8 indicated cows that were in metestrus on d 0 or had ovulated while carrying sham devices (early CL).

Behavioral Response to Treatment. To detect onset of behavioral estrus, cows were observed for at least 30 min twice daily at approximately 12-h intervals from initiation of treatment (d 0) to d 22 of the experiment. Data from cows were placed into one of three categories according to behavioral activity: 1) standing estrus, receptive to mounting by other cows; 2) active, cows exhibited sexual activity but would not stand to be mounted; or 3) no estrus, cows did not exhibit any signs of behavioral estrus.

Statistical Analyses. Data regarding ovulatory response of cows to treatment as indicated by concentrations of P_4 in circulation as well as the behavioral response of cows to treatment were fitted to a categorical data model (SAS, 1989) containing the fixed effects of cow, P_4 , EB, body condition score, location, parity, number of days during the postpartum period, $P_4 \times EB$, location $\times P_4$, and location $\times EB$. The Addcell option in PROC CATMOD was used to allow analysis of unbalanced data (SAS, 1989). Only data from d 0 to 11 of the experiment regarding behavioral response to treatment were analyzed, with d 9 to 11 being the period when the majority of behavioral responses to treatment were expected to occur.

Conclusions drawn from this experiment can potentially alter current management scenarios of cow-calf producers. Producers are ultimately interested in the number of cows exhibiting estrous cycles at the onset of the breeding season and its effect on reproductive efficiency. Therefore, predicted data were compiled and analyzed in which numbers of cows that had

Table 1. Proportions of cows within each treatment that either formed a corpus luteum or did not initiate luteal function

Response ^b	Treatment ^a			
	P ₄ + EB	EB	P ₄	Control
Anestrus ^c	15/93 (16%)	29/86 (34%)	28/92 (30%)	31/91 (34%)
Typical lifespan CL ^d	66/93 (71%)	17/86 (20%)	51/92 (55%)	15/91 (16%)
Late CL ^e	0/93 (0%)	14/86 (16%)	0/92 (0%)	6/91 (7%)
Short-lived CL ^f	4/93 (4%)	10/86 (12%)	5/92 (5%)	26/91 (29%)
Early CL ^g	8/93 (9%)	16/86 (19%)	8/92 (9%)	13/91 (14%)

^aP₄ + estradiol benzoate (EB) = intravaginal device containing progesterone for 7 d plus 1 mg of EB 24 to 30 h after device removal; EB = sham device for 7 d plus 1 mg of EB 24 to 30 h after device removal; P₄ = intravaginal device containing progesterone for 7 d; Control = sham device for 7 d.

^bEffect of the following variables on distribution of cows within response categories: P₄, $P < .001$; EB, $P > .10$; P₄ × EB, $P > .10$.

^cCows remained anestrous.

^dCows formed a corpus luteum (CL) with a typical lifespan.

^eCows formed a CL beyond 4 d after progesterone withdrawal.

^fCows formed a short-lived CL.

^gCows were in metestrus at initiation of treatment or ovulated during treatment with a sham device.

formed CL by various criteria, reflecting effects of treatment and natural resumption of estrous cycles, were considered (Table 3). These predicted data were fitted to a categorical data model containing the fixed effect of treatment. Within each response category, contrast statements were used to compare proportions of cows in the control group to cows treated with either P₄ alone, EB alone, or a combination of P₄ and EB.

Results

Response to Treatment. The proportion of cows that formed CL with a typical lifespan increased ($P < .001$) in response to treatment with P₄ (Table 1), but location, body condition score, parity, and number of days during the postpartum period had no effect ($P > .10$). There were no interactions ($P > .10$) among treatments with P₄ and EB or location affecting formation of CL with a typical lifespan.

Behavioral Response to Treatment. The proportion of cows that exhibited estrus (i.e., standing estrus or estrual activity) from d 0 to 11 increased ($P < .05$) in response to P₄ treatment (Table 2). Similarly, EB increased ($P < .001$) the proportion of cows that exhibited estrus (Table 2). Body condition score, parity, and number of days during the postpartum period did not affect ($P > .10$) the proportion of cows that exhibited estrus. However, there was an effect ($P < .05$) of location on proportion of cows detected in estrus. There were no interactions ($P > .10$) between P₄ and EB, location and P₄, or location and EB for the incidence of estrus. The majority of cows that exhibited estrual activity did so from d 9 to 11 of the experiment; few cows exhibited estrual activity during the treatment period from d 0 to 8 (Figure 1; Table 2).

Predicted Proportions. A greater proportion ($P < .01$) of cows treated with P₄ alone or in combination

with EB were induced to form CL with a typical lifespan, compared with untreated cows (Table 3). This effect of P₄ was enhanced ($P < .01$) by EB, but EB alone tended to reduce ($P < .10$) the proportion of cows that formed either a short-lived CL or CL with a typical lifespan, compared with untreated cows (Table 3). The combination of P₄ and EB increased the proportion of cows that formed CL in response to or during treatment ($P < .01$) and the proportion that had formed CL by the end of the experiment ($P < .05$), compared with untreated cows (Table 3).

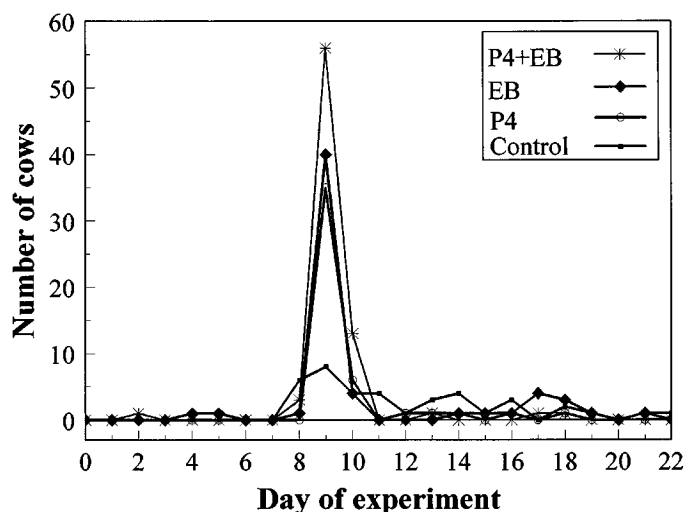


Figure 1. Number of cows within each treatment exhibiting estrual activity or standing estrus during the experiment. Cows were treated with either progesterone for 7 d (P₄), progesterone for 7 d plus 1 mg of estradiol benzoate (EB) 24 to 30 h after progesterone removal (P₄ + EB), a sham device for 7 d plus 1 mg of EB 24 to 30 h after device removal (EB), or a sham device for 7 d (Control).

Table 2. Proportions of cows within each treatment that exhibited standing estrus, were active, or did not exhibit signs of estrus

Behavioral response ^b	Treatment ^a			
	P ₄ + EB	EB	P ₄	Control
No estrus ^c	20/93 (22%)	39/86 (45%)	51/92 (55%)	69/91 (76%)
Formed a CL ^d	18/20 (90%)	28/39 (72%)	24/51 (47%)	41/69 (59%)
Standing estrus ^e	56/93 (60%)	33/86 (38%)	37/92 (40%)	17/91 (19%)
Formed a CL ^d	50/56 (89%)	20/33 (61%)	37/37 (100%)	15/17 (88%)
Active ^f	17/93 (18%)	14/86 (16%)	4/92 (4%)	5/91 (5%)
Formed a CL ^d	10/17 (59%)	9/14 (64%)	3/4 (75%)	4/5 (80%)
Estrous activity (standing + active)	73/93 (78%)	47/86 (55%)	41/92 (45%)	22/91 (24%)
Formed a CL ^d	60/73 (82%)	29/47 (62%)	40/41 (98%)	19/22 (86%)
d 0–8 ^g	4/73 (5%)	3/47 (6%)	0/41 (0%)	6/22 (27%)
d 9–11 ^g	69/73 (95%)	44/47 (94%)	41/41 (100%)	16/22 (73%)

^aP₄ + EB = intravaginal device containing progesterone for 7 d plus 1 mg of estradiol benzoate (EB) 24 to 30 h after device removal; EB = sham device for 7 d plus 1 mg of EB 24 to 30 h after device removal; P₄ = intravaginal device containing progesterone for 7 d; Control = sham device for 7 d.

^bEffect of the following variables on distribution of cows in response categories of no estrus, standing estrus, and active: P₄, $P < .05$; EB, $P < .001$; P₄ × EB, $P > .10$; Location, $P < .05$.

^cProportion of cows that did not exhibit signs of behavioral estrus.

^dProportions of cows that formed a corpus luteum (CL) during the experiment.

^eProportion of cows that exhibited standing estrus either during treatment or within 3 d after the end of treatment.

^fProportion of cows that exhibited signs of behavioral estrus but not standing estrus either during treatment or within 3 d after the end of treatment.

^gProportion of cows that were either active or exhibited standing estrus from d 0 to 8 or d 9 to 11 of the experiment.

Discussion

Formation of CL with a typical lifespan was induced in 51 of 92 cows treated for 7 d with only P₄ and in 66 of 93 cows given EB after the 7-d period of P₄ (Table 1). Treatments that induce resumption of estrous cycles in cows that are anestrus, as in the present experiment, can potentially provide considerable economic benefits for cow-calf enterprises. Extended periods of anestrus after calving contribute to the inability of cows to remain on a yearly calving interval. These long postpartum intervals of anestrus (90 d) eventually result in lowered net income for the enterprise, primarily because of high replacement rates due to failure of cows to conceive during the breeding season (Werth et al., 1991). Data from the present study reveal that short-term treatment of cows that are anestrus with P₄ can induce earlier ovulation in some cows, increase the percentage of cows exhibiting estrous cycles during the breeding season, and presumably increase the percentage of cows conceiving at first service.

Cows are anestrus during their postpartum period for two primary reasons: 1) lactational anestrus caused by a suckling stimulus or 2) nutritional anestrus caused by inadequate energy stores (Short et al., 1990). The present study focused on inducing estrous cycles in cows in lactational anestrus. We recognize that nutritional stresses may have been present in some cows because body condition scores ranged from 2.5 to 8. However, we observed no effect

of body condition score on formation of CL, possibly because the majority of cows had adequate body condition for reproductive functions. Nonetheless, caution should be used in applying the results of the present study to cows in nutritional anestrus because the ability of P₄ treatment to overcome the inhibitory effects of anestrus may differ between animals in nutritional and lactational anestrus. Treatment of cows that are anestrus with LHRH pulses during the postpartum period induced ovulation and CL formation, indicating that limited release of LHRH by the hypothalamus rather than the ability of the pituitary to secrete LH is likely the principal cause of lactational anestrus (Walters et al., 1982; Jagger et al., 1987). Others, however, observed that intermittent injections of LHRH to cows that are anestrus did not induce ovulation; this lack of response was attributed to inadequate body condition (Short et al., 1981). Cows in a nutritional state of anestrus seem to be in a “deeper” state of anestrus and are not as readily able to ovulate in response to treatment with pulses of LHRH.

Numerous treatment regimens have been tested to induce ovulation, formation of CL with typical lifespans, and behavioral estrus in cows and heifers that are anestrus. Treatments with progestins such as norgestomet were effective in inducing ovulation and behavioral estrus in heifers and cows that were anestrus (Miksch et al., 1978; Smith et al., 1987; Anderson et al., 1996). Additionally, treatment of beef cows with P₄ or norgestomet in combination with

GnRH or hCG during the postpartum period of anestrus induced ovulation and formation of CL with typical life spans (Sheffel et al., 1982; Smith et al., 1983, 1987). Suckled beef cows that were anestrous and implanted with an LHRH analog during the early postpartum period resumed estrous cycles earlier than control cows (Roberge et al., 1992). Dairy cows that were anestrous ovulated and exhibited behavioral estrus following treatment with PMSG and an intravaginal device containing P₄ (Macmillan and Peterson, 1993).

The mechanism by which treatment with a progestin induces ovulation in cows that are anestrous is not completely understood. Increased secretion of LH during and after progestin withdrawal has been reported for lactating cows that are anestrous (Garcia-Winder et al., 1987) and prepubertal heifers (Anderson and Day, 1996; Anderson et al., 1996). One unsubstantiated explanation for this increase in LH secretion that has been generally advanced is that short-term progestin treatment allows pituitary stores of LH to increase. Upon withdrawal of the progestin, LH secretion increases, which enhances estradiol production and maturation of ovarian follicles to the point that ovulation occurs. Alternatively, the increase in LH secretion may be the result of the actions of progestins to accelerate the prepubertal decline in estradiol negative feedback on LH (Anderson et al., 1996) through site-specific reductions in the number of neurons containing estradiol receptors in the hypothalamus (Anderson and Day, 1996). Because estradiol negative feedback has been demonstrated to

be a potent regulator of LH secretion in postpartum cows (Acosta et al., 1983), similar actions of progestins in postpartum cows are plausible.

Resumption of estrous cycles in postpartum cows is often characterized by the formation of CL with short life spans (Short et al., 1972; Humphrey et al., 1983). In the present study, 26 of 91 untreated cows formed a short-lived CL. Premature release of PGF_{2α} by the uterus is thought to cause the earlier demise of the CL (Copelin et al., 1989; Peter et al., 1989; Cooper et al., 1991). Treatment of cows with a progestin enhanced the number of P₄ receptors present in the uterus on d 5 of the estrous cycle (Zollers et al., 1993). Therefore, it is likely that endogenous P₄ secretion during a short luteal phase increases the number of P₄ receptors in the uterus and controls the release of PGF_{2α} during the subsequent estrous cycle so that the luteal phase is of typical length. Progestins such as melengesterol acetate (MGA) and norgestomet may function much like endogenous P₄ during a short luteal phase in properly timing the release of PGF_{2α} during the next estrous cycle so that the CL formed has a typical life span and function (Cooper et al., 1991). In the present experiment, cows given P₄ for 7 d formed fewer short-lived CL (9/185; 4.9%) than did control cows (26/91; 28.6%). The short-term treatment with P₄ may time the release of PGF_{2α} from the uterus so that the life of the CL is characteristic of a typical luteal phase.

Natural resumption of reproductive function in postpartum cows often occurs without signs of behavioral estrus preceding the initial increase in P₄

Table 3. Predicted proportions of cows within each treatment group that formed a corpus luteum by various criteria

Response	Treatment ^a			
	P ₄ + EB	EB	P ₄	Control
Formed or would have formed a CL during progesterone treatment ^b	7	8	7	5
Formed a CL with a typical lifespan ^c	59/78 (76%)**	17/70 (24%)	44/77 (57%)*	15/78 (19%)
Formed a short-lived or typical lifespan CL ^d	63/78 (81%)**	27/70 (39%) [†]	49/77 (64%)	41/78 (53%)
Total cows that formed a CL by 4 d after the end of treatment ^e	78/93 (84%)**	43/86 (50%)	64/92 (70%)	54/91 (59%)
Total cows that formed a CL by end of experiment ^f	78/93 (84%)*	57/86 (66%)	64/92 (70%)	60/91 (70%)

^aP₄ + estradiol benzoate (EB) = intravaginal device containing progesterone for 7 d plus 1 mg of EB 24 to 30 h after device removal; EB = sham device for 7 d plus 1 mg of EB 24 to 30 h after device removal; P₄ = intravaginal device containing progesterone for 7 d; Control = sham device for 7 d.

^bNumber of cows predicted to have formed a corpus luteum (CL) during treatment. Values for cows treated with sham devices were calculated from data for the early CL response in Table 1. For EB, sham device (16/86) minus progesterone device (8/93) = 8. For control, sham (13/91) minus progesterone (8/92) = 5. Value for progesterone-treated cows was estimated as the mean of these values (6.5, rounded to 7).

^cProportion of cows that formed a CL with a typical lifespan. This proportion excludes from the denominator cows that were in metestrus at treatment initiation or that formed or were predicted to form a CL while carrying a device (early CL response in Table 1).

^dProportion of cows that formed either a CL with a typical lifespan or a short-lived CL. The early CL response is excluded from the denominator.

^eProportion of cows in which a CL had formed by 4 d after the end of treatment.

^fProportion of cows that formed a CL by the end of the experimental period.

[†]Proportion differs from control: *P* < .10.

*Proportion differs from control: *P* < .05.

**Proportion differs from control. *P* < .01.

following parturition (Humphrey et al., 1983; Werth et al., 1996). Ovarian follicles destined to form CL with shortened lifespans, which are common during natural resumption of estrous cycles, secreted or contained less estradiol than follicles that formed CL with typical lifespans (Sheffel et al., 1982; Garcia-Winder et al., 1987). The absence of adequate LH during the period of anestrus may not allow the proper acquisition of LH receptors by thecal and granulosa cells, thereby resulting in reduced production of estradiol by preovulatory follicles (Inskeep et al., 1988). Additionally, reduced pulse frequency of LH during periods of anestrus may not provide a sufficient stimulus for estradiol production, thus leading to an inadequate preovulatory LH surge.

Providing an exogenous source of estradiol (i.e., EB) in the present experiment increased the proportion of cows that exhibited signs of behavioral estrus, and this estrual behavior was concentrated from d 9 to 11 of the experiment (Figure 1; Table 2). A greater proportion of cows treated with the combination of P₄ and EB were either active or exhibited standing estrus compared with cows treated with P₄ alone (Table 2). Regardless of treatment, the majority of cows exhibited standing estrus or were active from d 9 to 11 of the experiment (Figure 1; Table 2). Some of the cows in the control group were probably induced to initiate estrous cycles by the concentrated activity of estrus of treated cows; however, more control cows exhibited estrual activity beyond d 11 (Figure 1). It is well documented that endogenous estradiol produced by ovarian follicles enhances the amplitude of LH pulses during the follicular phase of the estrous cycle (Stumpf et al., 1989) by increasing numbers of LHRH receptors at the anterior pituitary (Schoenemann et al., 1985), thereby increasing the estrogenic capacity of preovulatory follicles. This positive feedback loop between estradiol and LH is critical in enabling an ovarian follicle to mature and ovulate and in facilitating expression of estrus coincident with ovulation. A greater proportion of cows treated with EB alone, however, exhibited estrus, but did not form CL, than of cows in all other treatment groups (Table 2). In the absence of P₄, we speculate that circulating concentrations of LH are not sufficient for development of a preovulatory follicle; however, a 1-mg dose of EB is sufficient to elicit behavioral signs of estrus.

Because estradiol induces the preovulatory LH surge, we expected that treatment with EB in addition to P₄ would further increase the proportion of cows forming CL following treatment. When data are analyzed as reported in Table 1, EB did not enhance the response of progesterone in inducing onset of luteal function. From a practical standpoint, however, producers are ultimately interested in the number of cows exhibiting estrous cycles at the onset of the breeding season. Having a greater percentage of the breeding herd exhibiting estrous cycles by the onset of the breeding season should enable more cows to

become pregnant early in the breeding season and fewer nonpregnant cows would result. Analyzing data as reported in Table 3 allows for the consideration that some cows would have initiated estrous cycles in the absence of progesterone treatment and compares the predicted effectiveness of each treatment with data for control cows in inducing luteal function by the end of the experiment. Based on the predicted data in Table 3, a greater proportion of cows treated with the combination of P₄ and EB would be expected to form CL that were short-lived or of typical lifespans, compared to the proportions for untreated control cows. Treatment with P₄ alone should increase the numbers of cows developing CL with typical or short lifespans (Table 3). The data in Table 3 indicate that treatment with the combination of P₄ and EB should be expected to be more effective in inducing luteal function by the end of an experiment than were the other treatments. Combining P₄ and EB treatments may induce an adequate preovulatory LH surge in a portion of cows with insufficient endogenous estradiol production, thus increasing the number of cows developing corpora lutea (Ulberg and Lindley, 1960).

Although the exact mechanism by which P₄ induces ovulation in cows during periods of anestrus is not clear, we speculate that increased secretion of LH either during or after P₄ treatment enhances ovarian follicular growth and estradiol production leading to ovulation. Treatment with EB following P₄ removal hastens or amplifies the preovulatory LH surge and expression of estrus. The combination of short-term treatment with P₄ and an injection of EB properly times the endocrine events necessary to increase the numbers of cows exhibiting estrous cycles during the postpartum period.

Implications

Initiation of estrous cycles in cows that are anestrus can potentially provide considerable economic benefit to cow-calf producers. Treating postpartum beef cows during lactational anestrus with progesterone and estradiol benzoate induced estrus and formation of corpora lutea with typical lifespans. These responses can increase the percentage of cows exhibiting estrous cycles at the onset of the breeding season and may result in more cows being maintained on a yearly calving interval and fewer cows being culled from the herd. Because these same treatments can be used to synchronize estrus in cows exhibiting estrous cycles, they provide the potential for artificial insemination of a large proportion of the herd at the same time.

Literature Cited

- Acosta, B., G. K. Tarnavsky, T. E. Platt, D. L. Hamernik, J. L. Brown, H. M. Schoenemann, and J. J. Reeves. 1983. Nursing enhances the negative effect of estrogen on LH release in the

- cow. *J. Anim. Sci.* 57:1530.
- Anderson, L. H., and M. L. Day. 1996. Site-specific reductions in the number of hypothalamic estradiol receptor-containing neurons during progestin-induced puberty in heifers. *Biol. Reprod.* 54: (Suppl. 1):178.
- Anderson, L. H., C. M. McDowell, and M. L. Day. 1996. Progestin-induced puberty and secretion of luteinizing hormone in heifers. *Biol. Reprod.* 54:1025.
- Bellows, R. A., R. E. Short, and R. B. Staigmiller. 1979. Research areas in beef cattle reproduction. In: H. Hawk (Ed.) *Beltsville Symposium Agricultural Research 3, Animal Reproduction*. pp 3-18. Allanheld, Osmun and Co. Publishers, New York.
- Bellows, R. A., R. B. Staigmiller, J. M. Wilson, D. A. Phelps, and A. Darling. 1991. Use of bovine FSH for superovulation and embryo production in beef heifers. *Theriogenology* 35:1069.
- Bergfeld, E.G.M., F. N. Kojima, A. S. Cupp, M. E. Wehrman, K. E. Peters, V. Mariscal, T. Sanchez, and J. E. Kinder. 1996. Changing dose of progesterone results in sudden changes in frequency of LH pulses and secretion of 17β -estradiol in bovine females. *Biol. Reprod.* 54:546.
- Brown, J. G., D. W. Peterson, and W. D. Foote. 1972. Reproductive response of beef cows to exogenous progestogen, estrogen and gonadotropins at various stages postpartum. *J. Anim. Sci.* 35: 362.
- Cooper, D. A., D. A. Carver, P. Villeneuve, W. J. Silvia, and E. K. Inskeep. 1991. Effects of progestagen treatment on concentrations of prostaglandins and oxytocin in plasma from the posterior vena cava of post-partum beef cows. *J. Reprod. Fertil.* 91:411.
- Copelin, J. P., M. F. Smith, D. H. Keisler, and H. A. Garverick. 1989. Effect of active immunization of pre-partum and post-partum cows against prostaglandin $F_{2\alpha}$ on lifespan and progesterone secretion of short-lived corpora lutea. *J. Reprod. Fertil.* 87:199.
- Garcia-Winder, M., P. E. Lewis, E. C. Townsend, and E. K. Inskeep. 1987. Effects of norgestomet on follicular development in postpartum beef cows. *J. Anim. Sci.* 64:1099.
- Humphrey, W. D., C. C. Kaltenbach, T. G. Dunn, D. R. Koritnik, and G. D. Niswender. 1983. Characterization of hormonal patterns in the beef cow during postpartum anestrus. *J. Anim. Sci.* 56: 445.
- Inskeep, E. K., T. D. Braden, P. E. Lewis, M. Garcia-Winder, and G. D. Niswender. 1988. Receptors for luteinizing hormone and follicle-stimulating hormone in largest follicles of postpartum beef cows. *Biol. Reprod.* 38:587.
- Jagger, J. P., A. R. Peters, and G. E. Lamming. 1987. Hormone responses to low-dose GnRH treatment in post-partum beef cows. *J. Reprod. Fertil.* 80:263.
- Macmillan, K. L., and A. J. Peterson. 1993. A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronisation, increasing pregnancy rates and the treatment of post-partum anoestrus. *Anim. Reprod. Sci.* 33:1.
- Macmillan, K. L., V. K. Taufa, D. R. Barnes, and A. M. Day. 1991. Plasma progesterone concentrations in heifers and cows treated with a new intravaginal device. *Anim. Reprod. Sci.* 26:25.
- Miksch, E. D., D. G. LeFeuer, G. Mukembo, J. G. Spitzer, and J. N. Wiltbank. 1978. Synchronization of estrus in beef cattle II. Effect of an injection of norgestomet and implant in heifers and cows. *Theriogenology* 10:201.
- Peter, A. T., W. K. Bosu, R. M. Liptrap, and E. Cummings. 1989. Temporal changes in serum prostaglandin $F_{2\alpha}$ and oxytocin in dairy cows with short luteal phases after the first postpartum ovulation. *Theriogenology* 32:277.
- Roberge, S., R. D. Schramm, A. V. Schally, and J. J. Reeves. 1992. Reduced postpartum anestrus of suckled beef cows treated with microencapsulated luteinizing hormone-releasing hormone analog. *J. Anim. Sci.* 70:3825.
- Saiduddin, S., M. M. Quevedo, and W. D. Foote. 1968. Response of beef cows to exogenous progesterone and estradiol at various stages postpartum. *J. Anim. Sci.* 27:1015.
- SAS. 1989. *SAS User's Guide: Statistics (5th Ed.)*. SAS Inst. Inc., Cary, NC.
- Schoenemann, H. M., W. D. Humphrey, M. E. Crowder, T. M. Nett, and J. J. Reeves. 1985. Pituitary luteinizing hormone-releasing hormone receptors in ovariectomized cows after challenge with ovarian steroids. *Biol. Reprod.* 32:574.
- Sheffel, C. E., B. R. Pratt, W. L. Ferrell, and E. K. Inskeep. 1982. Induced corpora lutea in the postpartum beef cow. II. Effects of treatment with progestogen and gonadotropins. *J. Anim. Sci.* 54:830.
- Short, R. E., R. A. Bellows, E. L. Moody, and B. E. Howland. 1972. Effects of suckling and mastectomy on bovine postpartum reproduction. *J. Anim. Sci.* 34:70.
- Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. *J. Anim. Sci.* 68: 799.
- Short, R. E., E. M. Convey, R. B. Staigmiller, and R. A. Bellows. 1981. Effects of intermittent small-dose injections of GnRH in anestrus postpartum beef cows. *J. Anim. Sci.* 53 (Suppl.):366 (Abstr.).
- Smith, M. F., A. W. Lishman, G. S. Lewis, P. G. Harms, M. R. Ellersieck, E. K. Inskeep, J. N. Wiltbank, and M. S. Amoss. 1983. Pituitary and ovarian responses to gonadotropin releasing hormone, calf removal and progestogen in anestrus beef cows. *J. Anim. Sci.* 57:418.
- Smith, V. G., J. R. Chenault, J. F. McAllister, and J. W. Lauderdale. 1987. Response of postpartum beef cows to exogenous progestogens and gonadotropin releasing hormone. *J. Anim. Sci.* 64:540.
- Stumpf, T. T., M. L. Day, J. A. Stotts, M. W. Wolfe, P. L. Pennel, R. J. Kittok, and J. E. Kinder. 1989. Effect of estradiol on luteinizing hormone secretion during the follicular phase of the bovine estrous cycle. *Biol. Reprod.* 41:91.
- Ulberg, L. C., and C. E. Lindley. 1960. Use of progesterone and estrogen in the control of reproductive activities in beef cattle. *J. Anim. Sci.* 19:1132.
- Walters, D. L., R. E. Short, E. M. Convey, R. B. Staigmiller, T. G. Dunn, and C. C. Kaltenbach. 1982. Pituitary and ovarian function in postpartum beef cows. III. Induction of estrus, ovulation and luteal function with intermittent small dose injections of GnRH. *Biol. Reprod.* 26:655.
- Werth, L. A., S. M. Azzam, M. K. Nielsen, and J. E. Kinder. 1991. Use of a simulation model to evaluate the influence of reproductive performance and management decisions on net income in beef production. *J. Anim. Sci.* 69:4710.
- Werth, L. A., J. C. Whittier, S. M. Azzam, G. H. Deutscher, and J. E. Kinder. 1996. Relationship between circulating progesterone with conception at the first postpartum estrus in young primiparous beef cows. *J. Anim. Sci.* 74:616.
- Zollers, W. G., H. A. Garverick, M. F. Smith, R. J. Moffatt, B. E. Salfen, and R. S. Youngquist. 1993. Concentrations of progesterone and oxytocin receptors in endometrium of postpartum cows expected to have a short or normal oestrous cycle. *J. Reprod. Fertil.* 97:329.