Induced and Synchronized Estrus in Cattle: Dose Titration of Estradiol Benzoate in Peripubertal Heifers and Postpartum Cows After Treatment with an Intravaginal Progesterone-Releasing Insert and Prostaglandin $F_{2\alpha}$[1,2]

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ABSTRACT: Peripubertal beef heifers (n = 57) and postpartum multiparous cows (n = 52) were used to determine the optimal dose of estradiol benzoate (EB) to induce and synchronize estrus after treatment with intravaginal progesterone inserts (IVP4, EAZI-BREED™ CIDR®). All females received an IVP4 for 7 d (d 0 = insertion day) with a 25-mg injection of $PGF_{2\alpha}$ (Lutalyse®) on d 6. At 24 to 30 h after IVP4 removal, females were randomly assigned to be injected subcutaneously with EB at the following doses: heifers 0, .2, .38, or .75 mg and cows 0, .25, .5, or 1 mg. Furthermore, seven heifers and seven cows from each dose group were bled every 4 h for 76 h starting at EB injection. Serum was collected and assayed for LH and estradiol-17β (E_2). Observations for signs of estrus were made twice daily for 21 d after removal of IVP4, and females were artificially inseminated 8 to 20 h after detection of estrus. The percentage of females showing estrous behavior was increased by EB ($P < .04$); the greatest response was at .38 mg in heifers (86%) and 1 mg in cows (100%). Dose × time interaction affected ($P < .01$) E_2 concentrations in heifers and cows; the animals that received the higher doses of EB had greater E_2 concentrations in a shorter time than those that received the smaller doses. The percentage of cows and heifers with an acute preovulatory LH release (peak LH) was affected by dose, with a linear ($P < .01$) and a quadratic ($P < .01$) response. Highest concentrations of LH during peak LH were affected by dose with a linear ($P < .01$) response in heifers and linear ($P < .01$) and quadratic ($P < .08$) responses in cows. Heifers receiving .38 mg and cows receiving .5 and 1 mg of EB had the highest peak LH. Time to LH peak had a linear ($P < .03$) response in heifers and had linear ($P < .04$) and quadratic ($P < .05$) responses in cows. Pregnancy rate was affected ($P < .02$) in heifers by whether or not they were anestrous before IVP4 treatment (those with estrous cycles = 52% vs those that were anestrous = 22%) and in cows by dose of EB ($P < .01$; 8, 23, 21, and 67% for 0, .25, .5, and 1 mg, respectively). In conclusion, in females treated with IVP4 and $PGF_{2\alpha}$ to induce and synchronize estrus, an injection of EB increased concentrations of E_2 and LH and increased number of animals showing estrus. Also, EB increased pregnancy rates in cows. Optimal responses were at .38 mg EB for heifers and at 1 mg EB for cows.

Key Words: Cattle, Estrus, Synchronization, Progesterone, Estradiol, Pregnancy Rate

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Introduction

Synchronization of estrus in cattle is an effective management tool, especially when using artificial insemination. Several products are used for estrus synchronization in cows and in heifers that have started estrous cycles, but most of these products are

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ineffective for anestrous cows and prepubertal heifers. Progestins have been shown to induce estrous cycles in anestrous cows and prepubertal heifers (Short et al., 1976; Miksch et al., 1978; Smith et al., 1987; Anderson and Day, 1994). Cows ovulating after pretreatment with progestins during postpartum anestrus formed a corpus luteum (**CL**) with a normal life span (Ramirez Godinez et al., 1981; Sheffel et al., 1982; Cooper et al., 1991). A single injection of estradiol-17β during the postpartum period hastened the onset of estrus and ovulation but reduced conception rates (Saiduddin et al., 1968). However, when an injection of estradiol was given to heifers and cows 24 to 72 h after the end of a 9-to-14-d progesterone treatment, estrous behavior and ovulation were induced without decreasing pregnancy rates (Ulberg and Lindley, 1960; Saiduddin et al., 1968; Brown et al., 1972). Recently, it was reported that in cows (Fike et al., 1997) and heifers (Johnson et al., 1997) treated with an intravaginal progesterone insert (**IVP4**) for 7 d plus an injection of estradiol benzoate (**EB**) 24 to 30 h after IVP4 removal, the EB increased the number of females exhibiting estrous behavior, compared with females not treated or treated with IVP4 only. Injections of EB will improve not only estrus and ovulation induction but also synchrony (Peters et al., 1977; Macmillan and Burke, 1996). We hypothesized that exogenous administration of EB 24 to 30 h after IVP4 removal could improve induction and synchrony of estrus, LH release, and ovulation and potentially increase pregnancy rates. The objectives of this trial were to determine the response to EB in this synchronization scheme and to determine the optimal dose of EB.

Materials and Methods

Crossbred (various crosses of Angus, Red Angus, Hereford, Simmental, Charolais, and Tarentaise) peripubertal heifers (n = 57, age = 11.8 to 13.6 mo) and postpartum (from d 30 to 60 after calving) multiparous cows (n = 52, age = 4 to 10 yr) were weighed and body condition-scored (**BCS**; 1 = emaciated to 9 = obese) on d –7 (d 0 = day cattle were given an IVP4 [EAZI-BREED™ CIDR®, InterAg, Hamilton, New Zealand]). Average weights and body condition scores were 310 kg and 5.5 for heifers and 536 kg and 4.7 for cows. Cows and heifers were bled from the tail on d –7 and 0 to classify each animal for estrous cycle status (**EC**; whether or not they had started estrous cycles) by monitoring changes in serum progesterone concentrations. Animals with progesterone concentrations ≥ 1 ng/mL at one or both bleedings were classified as having started estrous cycles (**ESTROUS CYCLES**), and those with progesterone concentrations < 1 ng/mL at both bleedings were classified as anestrous (**ANESTRUS**).

Table 1. Animal number distribution by age, estrous cycle status before estrus synchronization, and estradiol benzoate (EB) dose

Age and EB dose, mg	Estrous cycle status[a]: Estrous cycles	Estrous cycle status[a]: Anestrus	Total
Heifers			
0	4	10	14
.2	6	9	15
.38	6	8	14
.75	5	9	14
Total	21	36	57
Cows			
0	2	11	13
.25	2	11	13
.5	3	11	14
1	3	9	12
Total	10	42	52

[a]Determined by blood progesterone concentrations taken 7 d apart.

Animals were randomly assigned to one of four EB doses. Heifers were assigned by body weight and EC status; cows were assigned by age, calving date, and EC status (Table 1). Heifers and cows received an IVP4 for a 7-d period and a 25-mg injection of $PGF_{2\alpha}$ (Lutalyse®; Pharmacia & Upjohn, Kalamazoo, MI) on d 6. Twenty-four to 30 h after IVP4 removal, EB (.5 mg/mL of corn oil) was injected subcutaneously. Heifers received 0, .2, .38, or .75 mg and cows 0, .25, .5, or 1 mg of EB (Table 1).

Seven heifers and seven cows from each treatment were bled every 4 h via the tail or jugular vein for a 76-h period starting at EB injection. If a heifer or cow showed standing estrous behavior during sampling, blood collection stopped approximately 20 h after onset of estrus to decrease potential effects of stress on fertility. Blood samples were placed on ice immediately and stored at 5°C for 24 h until centrifugation. Serum was collected and stored at –20°C until assayed for LH and estradiol-17β. Serum LH concentrations were determined using a double antibody RIA as described by Price et al. (1987), and the intra- and interassay CV were 7.2 and 10.7%, respectively. Serum estradiol-17β concentrations were determined by J. S. Stevenson (Kansas State University, Manhattan) as described by Perry et al. (1991) and the intra- and interassay CV were 9.5 and 12.6%, respectively. Serum was collected from the blood samples collected on d –7 and 0 as previously described, and these were assayed for progesterone (Bellows et al., 1991) with intra- and interassay CV of 8.7 and 12.5%, respectively.

Cattle were observed for signs of estrus twice daily for a 21-d period starting on the day of IVP4 removal. Cows and heifers were artificially inseminated 8 to 20 h after detection of estrus and were only bred once during the 21-d time period. Pregnancy was determined using ultrasonography (Tokyo Keiki, LS-1000 with 5-MHz transducer) between 30 and 85 d after artificial insemination.

Data from heifers and cows were analyzed separately using GLM procedures of SAS (1994); the model included the dose of EB, the EC status, and the interaction of these two effects. The estradiol benzoate dose was used as a continuous class variable, and linear and quadratic dose of EB responses were tested by transforming doses + 1 to $\log_{10}$. When $F < 1.25$, that variable was deleted from the model. For hormone profiles, the repeated measures across time were included in the model as well as dose interactions with time. Discrete variables were coded 0 and 1 for analyses.

Results and Discussion

Estrous Behavior

The percentage of heifers showing estrous behavior within 120 h and 21 d after IVP4 removal (Table 2) was affected by dose of EB with a linear increase in response ($P < .04$). The response after 21 d was similar except that the increase with EB dose was nonlinear (quadratic; $P < .02$). The .38-mg dose of EB led to the greatest percentage of heifers that showed estrous behavior. The estrous behavior response to EB injection of postpartum cows indicated a linear increase response at 120 h ($P < .01$) and a linear ($P < .02$) and a quadratic ($P < .07$) at 21 d after IVP4 removal (Table 2). However, the response to dose differed with EC status (EC × dose; $P < .08$ and $P < .05$). The interaction resulted because the greatest response of ANESTRUS cows was at 1 mg EB, and the response of cows with ESTROUS CYCLES was 100% at .5 and 1 mg EB.

Heifers and cows receiving EB had a shorter time to onset of estrus ($P < .01$) than animals receiving no EB (Figure 1) during the first 120 h after IVP4 removal. In addition, among the EB-treated heifers that showed estrous activity, 89% showed onset of estrus between 36 to 48 h after IVP4 removal, and 100% of the EB-treated cows exhibited onset of estrus at 48 h. The tight synchrony of estrus in heifers and cows given EB

Table 2. Effects of estrous cycle status (anestrus vs estrous cycles) and dose of estradiol benzoate (EB) on the percentage (and proportion) of peripubertal heifers (n = 57) and postpartum cows (n = 52) that showed estrous behavior within 120 h or 21 d after the removal of an intravaginal progesterone insert, followed by an injection of one of four doses of EB

	120 h after insert removal			21 d after insert removal		
Age and EB dose, mg	Estrous cycles on d 0	Anestrus on d 0	Average	Estrous cycles on d 0	Anestrus on d 0	Average
Heifers						
0	25 (1/4)	50 (5/10)	43 (6/14)	50 (2/4)	50 (5/10)	50 (7/14)
.2	67 (4/6)	67 (6/9)	67 (10/15)	100 (6/6)	78 (7/9)	87 (13/15)
.38	100 (6/6)	75 (6/8)	86 (12/14)	100 (6/6)	88 (7/8)	93 (13/14)
.75	100 (5/5)	67 (6/9)	79 (11/14)	100 (5/5)	78 (7/9)	86 (12/14)
All doses	76 (16/21)	64 (23/36)	68 (39/57)	90 (19/21)	72 (26/36)	79 (45/57)
Probability						
Dose						
Linear			.04			.01
Quadratic			NS[a]			.02
Corpus luteum (CL)			—[b]			—[b]
Corpus luteum × dose			—[b]			—[b]
Pooled SE			.23			.20
Cows						
0	0 (0/2)	9 (1/11)	8 (1/13)	0 (0/2)	9 (1/11)	8 (1/13)
.25	50 (1/2)	55 (6/11)	54 (7/13)	50 (1/2)	73 (8/11)	69 (9/13)
.5	100 (3/3)	64 (7/11)	71 (10/14)	100 (3/3)	64 (7/11)	71 (10/14)
1	100 (3/3)	100 (9/9)	100 (12/12)	100 (3/3)	100 (9/9)	100 (12/12)
All doses	70 (7/10)	55 (23/42)	58 (30/52)	70 (7/10)	60 (25/42)	62 (32/52)
Probability						
Dose						
Linear			.01			.02
Quadratic			NS			.07
CL			NS			NS
CL × Dose			.08			.05
Pooled SE			.27			.26

[a]NS = not statistically significant.
[b]Deleted from model because $F < 1.25$.

24 to 30 h after IVP4 removal is supported by the endocrine profiles shown in Figure 2.

Beef cattle treated with exogenous progesterone for 9 d followed by an estradiol injection 48 h later were reported to have excellent estrous synchrony regardless of whether they were anestrous or had started estrous cycles (Ulberg and Lindley, 1960; Brown et al., 1972). Fike et al. (1997) and Johnson et al. (1997) reported an increase in number of postpartum cows and yearling heifers showing estrous behavior when given an estradiol injection 24 to 30 h after a 7-d IVP4 treatment.

Concentrations of Estradiol-17β in Serum

Concentrations of estradiol-17β in serum from heifers varied with time ($P < .01$) and the EB dose × time ($P < .01$) interaction and in cows with time ($P < .01$), EB dose ($P < .02$), and the EB dose × time ($P < .01$) interaction (Figure 2). From 4 to 28 h after EB injection, concentrations of estradiol-17β increased with increasing EB dose. However, by 28 h after the EB injection, serum estradiol-17β concentrations were similar among all groups (Figure 2).

Concentrations of estradiol-17β were increased by EB in heifers and cows from approximately 4 to 28 h after EB injection, which indicated that a single injection of EB could maintain increased concentrations of estradiol-17β for up over 24 h. Peak concentrations of estradiol-17β in heifers increased linearly with dose of EB ($P < .03$). However, the increase depended on EC status (EC × dose; $P < .04$; Table 3); concentrations were lower in ANESTRUS heifers than in those with ESTROUS CYCLES, except the effect was reversed for the highest dose. In cows, the increase in estradiol-17β was not significant ($P > .10$; Table 3). Time to peak estradiol-17β in serum in heifers was affected only by EC status ($P < .04$; Table 3); in cows, increasing EB dose resulted in a linear ($P < .02$; Table 3) reduction in time to peak estradiol-17β.

Short et al. (1973) reported that serum peak concentrations of estrogen occurred between 12 and 16 h after an injection of estradiol-17β in ovariectomized cows and that serum estrogen concentrations were increased for up to 28 h, which is nearly identical to the period of elevated serum estradiol-17β after EB in the present study.

Concentrations of LH in Serum

The proportion of heifers and cows that had an increase in concentrations of LH in serum as a result of EB treatment was affected by dose with a linear ($P < .01$) and a quadratic ($P < .01$) increase in response (Table 4). Peak concentrations of LH in heifers increased linearly ($P < .01$) with dose of EB. In cows, the changes in peak concentrations of LH with dose of EB were linear ($P < .01$) and quadratic ($P < .08$), but this response to EB differed between cows that were ANESTRUS and those that had started ESTROUS CYCLES (EC × dose; $P < .06$). This interaction resulted because cows that were ANESTRUS responded to a lower dose of EB and because cows that were ANESTRUS had greater response to the high

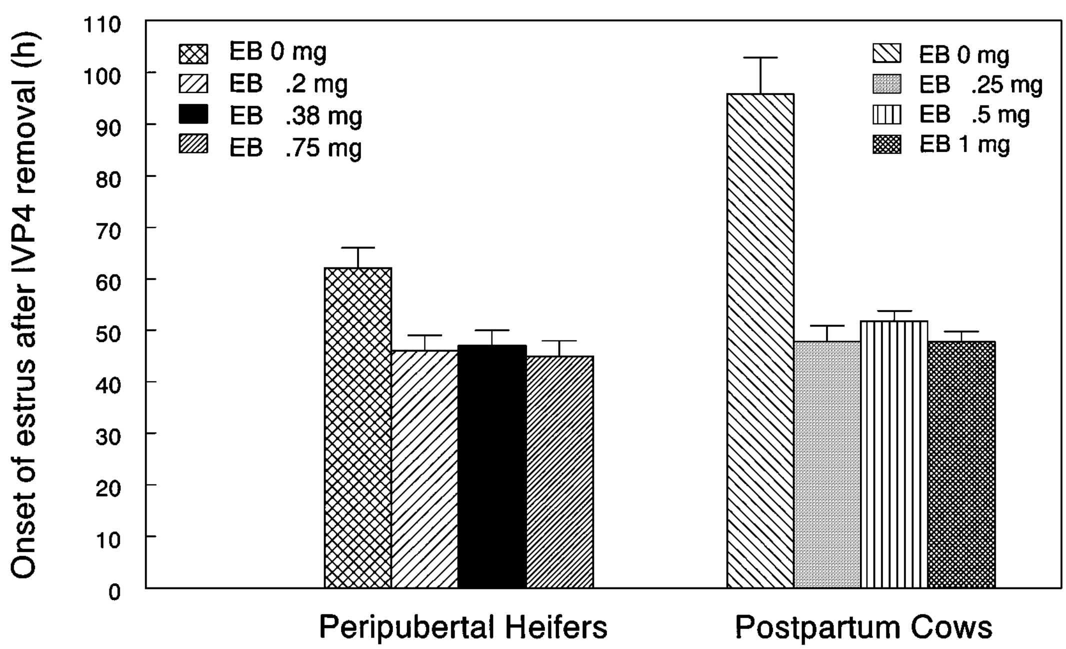

Figure 1. Time from removal of intravaginal progesterone (IVP4) insert to onset of estrus in peripubertal heifers and postpartum cows synchronized with an (IVP4) insert and $PGF_{2\alpha}$, followed by an injection of estradiol benzoate (EB) 24 to 30 h after insert removal as affected by EB dose ($P < .01$).

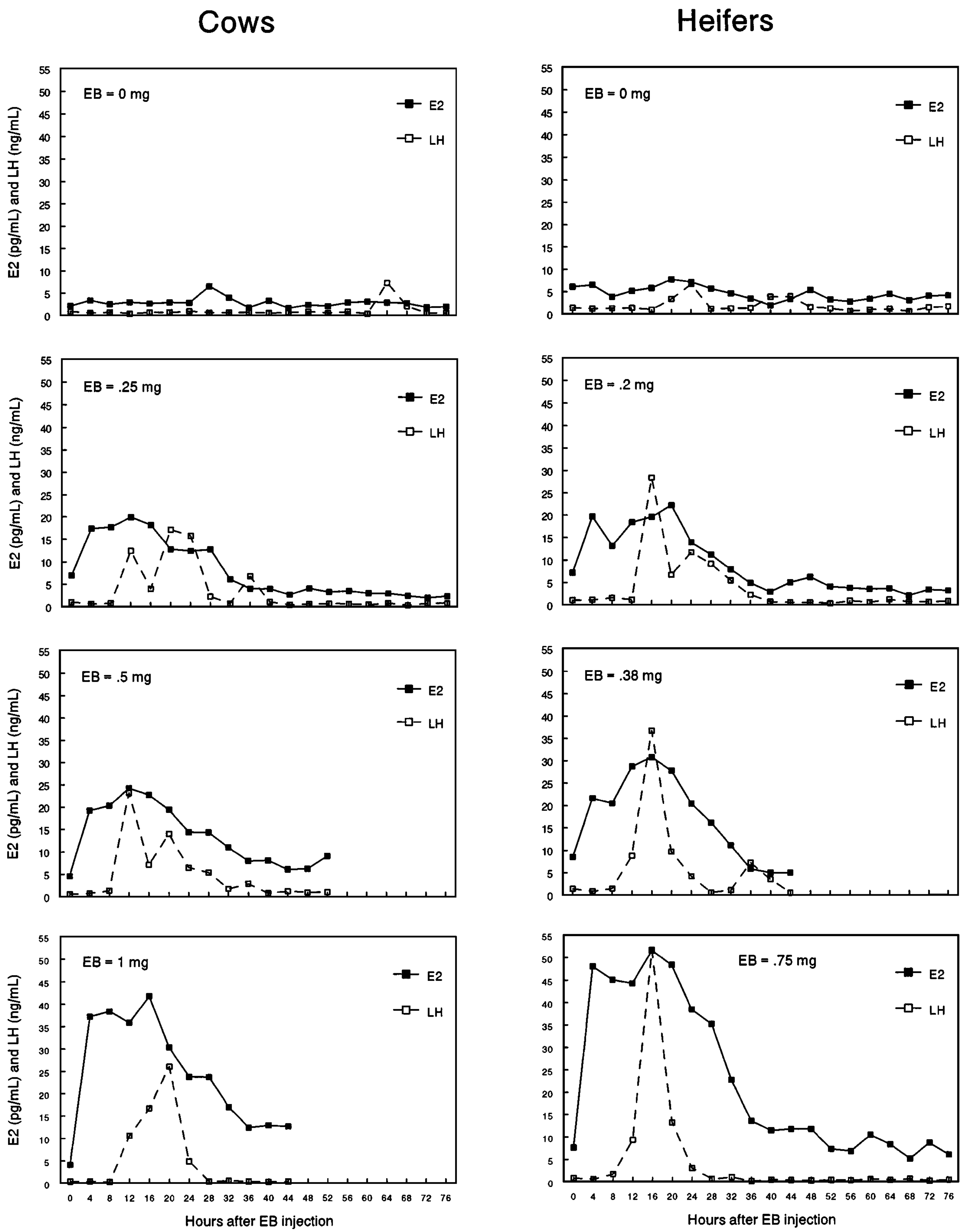

Figure 2. Serum estradiol-17β (E2) concentrations (SEM = 5.0) and estradiol benzoate (EB)-induced LH release (SEM = 8.4) in peripubertal heifers (n = 28) and postpartum cows (n = 27) synchronized with an intravaginal progesterone insert and an injection of $PGF_{2\alpha}$ as affected by dose of EB (given 24 to 30 h after insert removal, $P < .01$) and EB dose $\times$ time ($P < .01$).

dose of EB than cows with ESTROUS CYCLES. However, the intervals to peak concentrations of LH in serum were not affected by dose of EB (Table 4). Cows with ESTROUS CYCLES before estrus synchronization had lower serum peak LH concentrations than cows that were ANESTRUS (Table 4). Cows that were ANESTRUS before estrus synchronization had a similar interval to serum peak LH concentrations than cows with ESTROUS CYCLES (Table 4).

A rise in endogenous estrogen concentrations normally stimulates the preovulatory LH release in cows (Christensen et al., 1971; Henricks et al., 1971). Exogenous administration of estrogens also induced release of LH in cows (Short et al., 1973) and release of LH and FSH in prepubertal beef heifers (Gonzalez-Padilla et al., 1975), in agreement with the results in the present study. Furthermore, we reported that an EB dose as low as .2 in heifers and .25 mg in cows was sufficient to induce the LH surge, which supports results published by Short et al. (1979), who indicated that a low dose of .25 mg of estradiol-17β was sufficient to induce the LH surge in ovariectomized and postpartum anestrous cows. Among postpartum cows (Fike et al., 1997) and yearling heifers (Johnson et al., 1997) given IVP4 for 7 d and an EB injection 24 to 30 h after IVP4 removal, there was a significant increase in the proportion forming a functional CL. Based on results that all EB-treated animals sampled had an LH surge, we suggest that estradiol injection 24 to 30 h after IVP4 removal induces a preovulatory surge of LH and ovulation in postpartum cows and peripubertal heifers.

Pregnancy Rates

Pregnancy rates after breeding for 120 h ($P < .02$) or 21 d ($P < .08$) after IVP4 removal were higher in heifers with ESTROUS CYCLES than in heifers that were ANESTRUS (Table 5). Otherwise, there were no significant main effects or interactions affecting ($P >$ .10) pregnancy rates of heifers. Even though EB increased the proportion of heifers showing estrus after IVP4 removal, pregnancy rates were unaffected by dose of EB. The fact that EB did not affect pregnancy rates could be caused by 1) growth and dominance of follicles after EB that may have resulted in a new ovarian follicular wave caused by premature release of LH, which in turn interfered with pregnancy; 2) a premature release of LH that may have

Table 3. Effects of estradiol benzoate (EB) doses given 24 to 30 h after the removal of an intravaginal progesterone insert on serum peak estradiol-17β concentrations and time from EB injection to serum peak estradiol-17β in peripubertal heifers (n = 28) and postpartum cows (n = 27)

	Estradiol-17β peak, pg/mL			Time to estradiol-17β peak, h		
Age and EB dose, mg	Estrous cycles on d 0	Anestrus on d 0	Average	Estrous cycles on d 0	Anestrus on d 0	Average
Heifers						
0	10.8	8.1	9.4	22.0	17.6	19.8
.2	29.3	22.7	26.0	17.3	11.0	14.2
.38	42.4	31.7	37.0	16.0	17.0	16.5
.75	51.1	74.8	62.9	18.7	7.0	12.8
All doses	33.4	34.3	33.8	18.5	13.1	15.8
Probability						
Dose						
Linear			.03			—[a]
Quadratic			NS[b]			—[a]
Corpus luteum (CL)			NS			.04
Corpus luteum × dose			.04			NS
Pooled SE			8.1			4.8
Cows						
0	18.1	5.9	12.0	28.0	36.0	32.0
.25	29.9	22.2	26.1	12.0	17.3	14.7
.5	25.1	29.4	27.3	12.0	13.6	12.8
1	40.5	53.3	46.9	12.0	10.0	11.0
All doses	28.4	27.7	28.0	16.0	19.2	17.6
Probability						
Dose						
Linear			NS			.02
Quadratic			NS			NS
CL			NS			—[a]
CL × dose			—[a]			—[a]
Pooled SE			10.2			10.6

[a]Deleted from model because $F < 1.25$.
[b]NS = not statistically significant.

luteinized the dominant follicle so that ovulation did not occur; or 3) failure of ovulation because EB may have induced estrous behavior and LH release without development of a preovulatory follicle. Adams (1994) demonstrated that an injection of estradiol valerate given during the early-growing ovarian follicular phase terminated the growth of the dominant follicle and shortened the period of functional dominance. However, when estradiol valerate was given in the middle or at the end of the growing ovarian follicular phase, suppression of the dominant follicle growth and dominance did not occur. Refsal et al. (1987) suggested that estradiol-induced LH release could result in ovulation or in luteinization of the dominant follicle.

Pregnancy rate in postpartum cows was linearly affected ($P < .01$) by EB dose. Cows receiving the highest dose of EB (1 mg) had the highest pregnancy rate after IVP4 removal (Table 5). In addition, of the cows that received the 1-mg dose of EB and were pregnant, 88% were pregnant from an insemination within 48 h after IVP4 removal. These pregnancy rates were attained in cows in which 81% were anestrous at the beginning of the trial, which indicated that 1 mg of EB 24 to 30 h after IVP4 removal induced a fertile estrus in postpartum anestrous cows. These findings support those of Ulberg and Lindley (1960), Saiduddin et al. (1968), Brown et al. (1972), and Fike et al. (1997), who reported that progesterone treatment followed 24 to 72 h later by an injection of estradiol successfully induced a fertile estrus in heifers and cows.

In summary, a low dose of EB in heifers and cows induced estrous behavior and increased serum estradiol-17β concentrations. In general, the dose of EB linearly increased the preovulatory release of LH, proportion of cattle showing estrus, peak estradiol-17β concentrations, proportion of females having preovulatory release of LH, and peak LH concentrations, and the EB dose reduced the interval from IVP4 removal to LH peak. We conclude that the effectiveness of estrus synchronization in peripubertal heifers and

Table 4. Effects of estradiol benzoate (EB) doses given 24 to 30 h after the removal of an intravaginal progesterone insert on percentage (and proportion) of peripubertal heifers (n = 28) and postpartum cows (n = 27) showing acute preovulatory LH release, serum LH concentrations, and time from EB injection to serum LH peak

	% with elevated LH release			Peak serum LH concentrations, ng/mL			Time to serum LH peak, h		
Age and EB dose, mg	Estrous cycles on d 0	Anestrus on d 0	Average	Estrous cycles on on d 0	Anestrus on d 0	Average	Estrous cycles on d 0	Anestrus on d 0	Average
Heifers									
0	50 (1/2)	20 (1/5)	28 (2/7)	11.6	8.1	9.8	46.0	26.4	36.2
.2	100 (3/3)	100 (4/4)	100 (7/7)	44.6	47.7	46.1	25.3	26.0	25.7
.38	100 (3/3)	100 (4/4)	100 (7/7)	51.4	40.2	45.8	18.7	26.0	22.3
.75	100 (3/3)	100 (4/4)	100 (7/7)	74.8	59.2	67.0	20.0	20.0	20.0
All doses	91 (10/11)	76 (13/17)	82 (23/28)	45.6	38.8	42.2	27.5	24.6	26.0
Probability									
Dose									
Linear			.01			.01			.03
Quadratic			.01			NS			NS
Corpus luteum (CL)			NS[a]			—[b]			NS
CL × dose			—[b]			—[b]			.09
Pooled SE			.18			14.8			6.5
Cows									
0	0 (0/1)	0 (0/6)	0 (0/7)	1.3	1.2	1.3	12.0	21.3	16.7
.25	100 (1/1)	100 (6/6)	100 (7/7)	5.6	45.6	25.6	28.0	25.3	26.7
.5	100 (2/2)	100 (5/5)	100 (7/7)	45.8	48.1	46.9	28.0	28.0	28.0
1	100 (2/2)	100 (4/4)	100 (6/6)	33.2	66.3	49.8	24.0	19.0	21.5
All doses	83 (5/6)	71 (15/21)	74 (20/27)	21.5	40.3	30.9	23.0	23.4	23.2
Probability									
Dose									
Linear			.01			.01			.04
Quadratic			.01			.08			.05
CL			—[b]			.06			—[b]
CL × dose			—[b]			.06			—[b]
Pooled SE			.13			20.2			12.3

[a]NS = not statistically significant.
[b]Deleted from model because $F < 1.25$.

Table 5. Percentage (and proportion) of peripubertal heifers (n = 57) and postpartum cows (n = 52) that were pregnant at 120 h and 21 days after the removal of an intravaginal progesterone insert

	120 h after insert removal			21 d after insert removal		
Age and EB dose, mg	Estrous cycles on d 0	Anestrus on d 0	Average	Estrous cycles on d 0	Anestrus on d 0	Average
Heifers						
0	50 (2/4)	30 (3/10)	35 (5/14)	50 (2/4)	40 (4/10)	43 (6/14)
.2	67 (4/6)	11 (1/9)	33 (5/15)	100 (6/6)	44 (4/9)	67 (10/15)
.38	33 (2/6)	38 (3/8)	35 (5/14)	67 (4/6)	63 (5/8)	64 (9/14)
.75	60 (3/5)	11 (1/9)	29 (4/14)	80 (4/5)	56 (5/9)	64 (9/14)
All doses	52 (11/21)	22 (8/36)	33 (19/57)	76 (16/21)	50 (18/36)	60 (34/57)
Probability						
Dose						
Linear			—[a]			—[a]
Quadratic			—[a]			—[a]
Corpus luteum (CL)			.02			.08
CL × dose			—[a]			—[a]
Pooled SE			.24			.25
Cows						
0	0 (0/2)	9 (1/11)	8 (1/13)	0 (0/2)	9 (1/11)	8 (1/13)
.25	0 (0/2)	27 (3/11)	23 (3/13)	0 (0/2)	27 (3/11)	23 (3/13)
.5	33 (1/3)	18 (2/11)	21 (3/14)	33 (1/3)	18 (2/11)	21 (3/14)
1	33 (1/3)	67 (6/9)	58 (7/12)	33 (1/3)	78 (7/9)	67 (8/12)
All doses	20 (2/10)	29 (12/42)	27 (14/52)	20 (2/10)	31 (13/42)	36 (15/52)
Probability						
Dose						
Linear			.01			.01
Quadratic			—[a]			—[a]
CL			—[a]			—[a]
CL × dose			—[a]			—[a]
Pooled SE			.23			.23

[a]Deleted from model because $F < 1.25$.

postpartum cows using IVP4 and $PGF_{2\alpha}$ can be improved by an injection of EB at 24 to 30 h after IVP4 removal. The minimal effective dose of EB depends on the end point but in general seems to be approximately .4 mg in heifers and 1 mg in cows.

Implications

Many estrus synchronization programs available to producers have not been documented to be effective in cattle that have not started estrous cycles. We have shown that synchronization of estrus using a combined treatment of an intravaginal progesterone for 7 d, an injection of prostaglandin $F_{2\alpha}$ on d 6, and an injection of estradiol benzoate 24 to 30 h after removal of an intravaginal progesterone insert increased estrus detection and degree of estrus synchrony, even if the cattle had not started estrous cycles at the beginning of treatment. This combined program may increase the effectiveness of estrus synchronization especially in anestrous females, so that artificial insemination and timed insemination can be more easily used in beef cattle.

Literature Cited

Adams, G. P. 1994. Control of ovarian follicular wave dynamics in cattle: Implications for synchronization & superstimulation. Theriogenology 41:19–24.

Anderson, L. H., and M. L. Day. 1994. Progestin-induced puberty and LH secretion in heifers. Biol. Reprod. 50(Suppl. 1):86.

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 35:1069–1082.

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: 362–369.

Christensen, D. S., J. N. Wiltbank, and M. L. Hopwood. 1971. Blood hormone levels during the bovine estrous cycle. J. Anim. Sci. 33:251.

Cooper, D. A., D. A. Carver, P. Villeneuve, W. J. Silvia, and E. K. Inskeep. 1991. Effects of progestogen treatment on concentrations of prostaglandin and oxytocin in plasma from the posterior vena cava of postpartum beef cows. J. Reprod. Fertil. 91:411–421.

Fike, K. E., M. L. Day, E. K. Inskeep, J. E. Kinder, P. E. Lewis, R. E. Short, and H. D. Hafs. 1997. 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 benzoate. J. Anim. Sci. 75: 2009–2015.

Gonzalez-Padilla, E., G. D. Niswender, and J. N. Wiltbank. 1975. Puberty in beef heifers. II. Effect of injections of progesterone and estradiol-17β on serum LH, FSH and ovarian activity. J. Anim. Sci. 40:1105–1109.

Henricks, D. M., J. F. Dickey, and J. R. Hill. 1971. Plasma estrogen and progesterone levels in cows prior to and during estrus. Endocrinology 89:1350–1355.

Johnson, S. K., M. L. Day, J. M. Lynch, J. E. Kinder, R. Rasby, R. E. Short, R. P. Wettemann, and H. D. Hafs. 1997. Onset of estrus and luteal function in peripubertal heifers given an intravaginal progesterone releasing insert with or without a subsequent injection of estradiol benzoate. J. Anim. Sci. 75(Suppl. 1):231 (Abstr.).

Macmillan, K. L., and C. R. Burke. 1996. Effects of oestrous cycle control on reproductive efficiency. Anim. Reprod. Sci. 42: 307–320.

Miksch, E. D., D. G. LeFever, 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 10:201–210.

Perry, R. C., L. R. Corah, G. H. Kiracofe, J. S. Stevenson, and W. E. Beal. 1991. Endocrine changes and ultrasonography of ovaries in suckled beef cows during resumption of postpartum estrous cycles. J. Anim. Sci. 69:2548–2255.

Peters, J. B., J. A. Welch, J. W. Lauderdale, and E. K. Inskeep. 1977. Synchronization of estrus in beef cattle with $PGF_{2\alpha}$ and estradiol benzoate. J. Anim. Sci. 45:230–235.

Price, C. A., B. A. Morris, and R. Webb. 1987. Reproductive and endocrine effects of active immunization against a testosterone conjugate in the heifer. J. Reprod. Fertil. 81:149–160.

Ramirez Godinez, J. A., G. H. Kiracofe, R. M. McKee, R. R. Schalles, and R. J. Kittok. 1981. Reducing the incidence of short estrous cycles in beef cows with norgestomet. Theriogenology 15: 613–623.

Refsal, K. R., J. H. Jarrin-Maldonado, and R. F. Nachreiner. 1987. Endocrine profiles in cows with ovarian cysts experimentally induced by treatment with exogenous estradiol or adrenocorticotropic hormone. Theriogenology 28:871–889.

Saiduddin, S., M. M. Quevedo, and W. D. Foote. 1968. Response of beef cows to exogenous progesterone hormone and estradiol at various stages postpartum. J. Anim. Sci. 27:1015–1020.

SAS. 1994. SAS User's Guide: Statistics (Version 6.10 Ed.). SAS Inst. Inc., Cary, NC.

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. 54:830–836.

Short, R. E., R. A. Bellows, J. B. Carr, R. B. Staigmiller, and R. D. Randel. 1976. Induced or synchronized puberty in heifers. J. Anim. Sci. 43:1254–1258.

Short, R. E., B. E. Howland, R. D. Randel, D. S. Christensen, and R. A. Bellows. 1973. Induced LH release in spayed cows. J. Anim. Sci. 37:551–557.

Short, R. E., R. D. Randel, R. B. Staigmiller, and R. A. Bellows. 1979. Factors affecting estrogen-induced LH release in the cow. Biol. Reprod. 21:683–689.

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: 540–551.

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. 19:1132–1142.