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LUTEAL FUNCTION AND ESTRUS IN PERIPUBERTAL BEEF HEIFERS TREATED WITH AN INTRAVAGINAL PROGESTERONE RELEASING DEVICE WITH OR WITHOUT A SUBSEQUENT INJECTION OF ESTRADIOL

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ABSTRACT

The objectives of this experiment were to determine if treatment of beef heifers with progesterone (P_4) using an intravaginal device alone or in combination with estradiol benzoate (EB) would induce estrus and cause development of corpora lutea (CL) with a typical life span. Peripubertal heifers ($n=311$) were used when about 40% of the heifers had a functional CL. The heifers were assigned to receive one of the following treatments on Day 0: 1) a sham device for 7 d (C, $n=108$); 2) an intravaginal device containing P_4 for 7 d (P, $n=102$); or 3) an intravaginal device containing P_4 for 7 d plus an injection of 1 mg EB 24 to 30 h after device removal (PE, $n=101$). Serum concentrations of P_4 were determined on Days -7, 0, 8, 15 and 22. Weight and age of the heifers at the start of the trial averaged 292 ± 45 kg and 365 ± 38 d, respectively. A greater ($P < 0.0001$) proportion of the heifers from the PE than P group was in standing estrus (81 vs 37%) and formed normal CL (68 vs 44%) after device removal. Of the heifers exhibiting estrus, a greater ($P < 0.05$) proportion of PE (94%) than P (80%) heifers was active 1 to 3 d after implant removal. Short-term progesterone treatment increased the proportion of heifers in estrus and those forming normal CL, and adding EB to the progesterone treatment further enhanced these responses.

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INTRODUCTION

Many factors influence attainment of puberty in beef heifers. When puberty is not attained before the start of the breeding season, fertility (5) and potential income to the cow/calf producer is reduced (22). Treatments to induce puberty in heifers increase reproductive efficiency by allowing heifers to complete their sub-fertile first estrus before the start of the breeding season. These heifers therefore can be bred early in the breeding season and calve early in the calving season, thus resulting in greater weaning weights of their calves and in improved rebreeding performance.

Treatment with progestins induced estrous cycles in anestrus cows (9, 19) and induced puberty in heifers (1, 18). Treating beef cattle with EB following P_4 treatment induces ovulation (4, 12). Thus, the objectives of this experiment with peripubertal heifers were 1) to establish if a 7-d treatment of peripubertal beef heifers with P_4 alone or with P_4 plus EB initiates estrous cycles, as indicated by development of a functional CL, and 2) to determine the synchrony of estrus after treatment with P_4 alone or in combination with EB.

MATERIALS AND METHODS

Peripubertal cross-bred beef heifers ($n=311$) at 6 locations (Kansas, Montana, Nebraska [2], Ohio and Oklahoma) were used in this study. Each location served as a replicate. At each location, heifers were weighed and assigned a body condition score (1=emaciated; 9=obese; 21) on Day -7 of the experiment and were blocked across 3 treatments based on age, body condition score, weight and genotype. All heifers at each location were managed together. Each replicate was targeted to begin when about 40% of the heifers had P_4 concentrations in the serum of greater than 1 ng/mL. On Day 0 of the experiment, heifers received 1 of 3 treatments: 1) a sham intravaginal device for 7 d (C, $n=108$); 2) an intravaginal device containing P_4 (EAZI-BREED CIDR, InterAg, Hamilton, New Zealand) for 7 d (P, $n=102$); or 3) an intravaginal device containing P_4 for 7 d plus an injection of 1 mg of estradiol benzoate (CIDIROL, InterAg) 24 to 30 h after device removal (PE, $n=101$). The intravaginal device contained 1.9 g of P_4 , resulting in P_4 amounts that typically are reported in blood during the luteal phase of the estrous cycle in cattle (15). Treatment with estradiol benzoate alone was not included in the study, because this treatment failed to initiate estrous cycles in postpartum anestrus beef cows (9).

Blood samples were collected on Days -7, 0, 8, 15 and 22 (Day 0 being the day the intravaginal devices were inserted) via the jugular or tail vein and were stored at 4°C for approximately 24 h until centrifugation. Serum was decanted and stored at -20°C until assayed for concentration of P_4 . Progesterone assays from Kansas (Diagnostic Products, Los Angeles, CA), Montana (2), Nebraska (3), Ohio (1) and Oklahoma (23) had inter- and intra-assay CV's less than 14 and 9%, respectively. Heifers with P_4 serum concentrations ≥ 1 ng/mL on Day -7 and/or Day 0 were considered pubertal and were excluded from further analysis.

Luteal function in heifers throughout the experiment was assessed by monitoring changes in serum concentrations of P_4 . Pubertal heifers were grouped into luteal function response categories based on concentrations of P_4 in serum collected on Days 0, 8, 15 and 22 as follows: 1) serum P_4 increase of no more than 0.5 ng/mL on Days 0, 8, 15 or 22 indicated heifers were still

prepubertal (no CL); 2) P_4 concentrations less than 0.5 ng/mL on Days 0 and 8 followed by an increase of at least 0.5 ng/mL on Day 15 and remaining 0.5 ng/mL above pretreatment values on Day 22 indicated that heifers ovulated no later than 4 d after device removal and developed a functional CL (normal CL); 3) P_4 concentrations less than 0.5 ng/mL on Days 0 and 8 followed by an increase of at least 0.5 ng/mL on Day 15 but then decreasing to less than 0.5 ng/mL by Day 22 indicated that a CL was developed and a “short” estrous cycle had occurred (short CL); 4) P_4 concentrations less than 0.5 ng/mL on Days 0 and 8 followed by an increase of no more than 0.5 ng/mL on Day 15 but then P_4 concentrations increased more than 0.5 ng/mL on Day 22 indicated that heifers ovulated after Day 4 and formed a functional CL (late CL); and 5) P_4 concentrations less than 0.5 ng/mL on Day 0 followed by an increase of at least 0.5 ng/mL on Day 8 indicated that heifers were in metestrus on Day 0 or ovulated while carrying the device (early CL). Puberty was considered to have occurred in heifers in response Categories 2, 3 and 4.

To detect behavioral estrus, heifers were observed for at least 30 min twice daily at approximately 12 h intervals beginning on Day 0 (initiation of treatment) to Day 22 of the experiment. Estrual activity was classed into 1 of the following 3 categories: 1) standing estrus (standing to be mounted); 2) active estrus (exhibiting some signs such as mucous production or vaginal discharge) but not standing to be mounted; or 3) no signs of behavioral estrus.

Intravaginal device retention by heifers was 98.1%. Heifers that did not retain their device during the treatment period were excluded from the analysis.

Ovarian activity (concentration of P_4 in serum and behavioral response) was fitted to a categorical data model (SAS User's Guide, Statistics, Cary, NC) containing the fixed effects of treatment, weight, age, body condition, location and the interactions of the fixed effects. Two orthogonal, single degree of freedom contrasts were made, one for the effects of P_4 treatment (C vs P + PE), the other for the added effects of estradiol (P vs PE). The Addcell option in Proc Catmod was used to allow for unbalanced data.

A table of predicted data was compiled and analyzed in which numbers of heifers that had formed CLs by various criteria, accounting for both the effects of treatment and natural resumption of estrous cycles. These predicted data were fitted to a categorical data model containing the fixed effect of treatment. Within each category, contrast statements were used to compare proportions of heifers treated with either P or PE.

RESULTS

Age, weight, body condition and geographic location did not interact ($P > 0.10$) with treatment. Average age, weight and body condition score at the beginning of the experiment was 365 ± 38 d, 292 ± 45 kg and $5.1 \pm .70$, respectively.

The overall analysis revealed that P_4 or $P_4 + EB$ affected ($P < 0.01$) the distribution of heifers that were anestrous or developed normal, late, short or early CL (Table 1). Treatments increased ($P < 0.001$) the proportion of heifers that developed normal CL and reduced ($P < 0.001$) the proportion with a short estrous cycle (Table 1). Similarly, P_4 or $P_4 + EB$ affected ($P < 0.05$) the distribution of heifers not exhibiting estrus, those in standing estrus, or those demonstrating signs of active estrus (Table 2). Treatments increased ($P < 0.001$) the proportion of heifers exhibiting estrus and reduced ($P < 0.001$) the proportion that expressed no signs of estrus.

Table 1. Corpus luteum (CL) function in peripubertal beef heifers given progesterone (P) or progesterone and estradiol (PE)

Treatment	Animals Treated n	CL Response ^a n (%)				
		No CL ^b	Normal CL ^b	Late CL	Short CL ^b	Early CL
Control	108	68(63) ^c	13(12) ^c	9(8)	5(5) ^c	13(12) ^d
P	102	43(42)	45(44)	11(11)	0(0)	3(3)
PE	101	17(17)	69(68)	13(13)	1(1)	1(1)

^aResponse is defined in the Material and Methods section.

^bProportion of P heifers differs from PE heifers: $P < 0.001$.

^cProportion of treated heifers differs from controls: $P < 0.001$.

^dProportion of treated heifers differs from controls: $P < 0.01$.

Table 2. Estrus in peripubertal beef heifers given progesterone (P) or progesterone plus estradiol (PE)

Treatment	Numbers of Heifers ^b	Estrous Response ^a n (%)				
		No Estrus ^d	Standing Estrus ^d	Active Estrus ^e	Active + Standing ^d	Formed CL (n/n) ^c
Control	95	55(58) ^f	19(20) ^f	21(22)	40(42) ^f	16/40 ^f
P	99	40(40)	37(37)	22(22)	59(60)	47/59
PE	100	8(8)	81(81)	11(11)	92(92)	77/92

^aResponses defined in Materials and Methods.

^bHeifers in the response category "early" (Table 1) are excluded.

^cNumerator = number of heifers with a CL; Denominator = number of heifers exhibiting either active or standing estrus.

^dProportion of P heifers differs from PE heifers: $P < 0.001$.

^eProportion of P heifers differs from PE heifers: $P < 0.05$.

^fProportion of treated heifers differs from controls; $P < 0.001$.

Orthogonal contrasts revealed more ($P < 0.01$) heifers given PE (68%) developed normal CL compared with heifers given P (44%, Table 1). Similarly, more ($P < 0.001$) heifers given PE (81%) were detected in standing estrus than heifers given P (37%) alone (Table 2). There were no differences among treatments in the proportion of heifers in standing estrus that formed CLs. Among heifers that exhibited estrous activity (standing or active), 94% given PE expressed estrus 1 to 3 d after device removal (Table 3, Figure 1), which was more ($P < 0.05$) than among heifers given P (80%) alone.

Table 3. Day that estrus was expressed for heifers given progesterone (P) or progesterone and estradiol (PE)

Treatment	Number of Heifers ^a	Day of estrus ^b %		
		0 to 7	8 to 10 ^c	11 to 22
Control	16	22 ^d	40 ^e	38 ^e
P	47	13	80	7
PE	77	5	94	1

^aIncludes only heifers that formed a corpus luteum.

^bDay 0 is the day the vaginal device was inserted; Day 8 is the day of estradiol benzoate injection.

^cProportion of P heifers differs from PE heifers: $P < 0.05$.

^dProportion of treated heifers differs from controls: $P < 0.05$.

^eProportion of treated heifers differs from controls: $P < 0.001$.

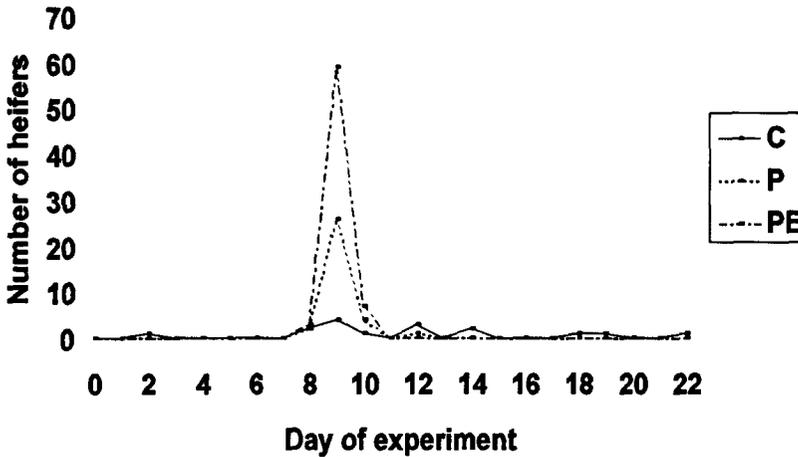


Figure 1. Number of heifers exhibiting standing estrus by day of the experiment. Heifers consisted of controls, those treated with progesterone (P₄) for 7 d (beginning on Day 0), or P₄ for 7 d plus 1 mg of estradiol (PE) on Day 8.

Conclusions drawn from this experiment can potentially alter current management protocols for heifers. Producers prefer that heifers exhibit estrous cycles before the beginning of the breeding season because this improves reproductive efficiency. The practical effects of treatments plus the natural commencement of estrous cycles is estimated in Table 4, which also lists the proportion of heifers within each treatment group that formed a CL by various criteria. Progesterone alone increased ($P < 0.01$) the proportion of the heifers with a normal CL from 14 to 39%, and the added estradiol treatment boosted ($P < 0.001$) the proportion to 65%. Within 15 d after device removal, P treatment alone increased ($P < 0.01$) the percentage of heifers with a CL from 37 to 58%, while estradiol treatment increased ($P < 0.001$) this rate to 83%.

Table 4. Proportion of heifers predicted to form a corpus luteum (CL) after treatment with progesterone (P) or progesterone plus estradiol (PE)

Treatment	Response, %				
	Formed CL or would have formed a CL while carrying the insert, n ^a	Formed fully functional CL ^{b,c}	Normal or short-lived CL ^{b,c}	Total heifers that formed CL by day 4 ^{c,d}	Total formed CL by day 22 ^{c,d}
Control	13	14 ^e	19 ^e	28 ^e	37 ^e
P	11	39	39	52	58
PE	11	65	66	81	83

^aValue for heifers was calculated from data in the response category "early" (Table 1). For control heifers: n=13.

^bDenominator excludes controls that formed a CL while carrying the device (response category; early, table 1) and treated heifers that would have formed a CL while carrying the device. Denominators are 95 for Control; 88 for P, and 89 for PE.

^cProportion of P heifers differs from PE; $P < 0.001$.

^dDenominator equal 108 for control, 102 for P, and 101 for PE; same as Table 1.

^eProportion of treated heifers differs from controls; $P < 0.01$.

DISCUSSION

While not all of the endocrine controls of puberty are clear, the hypothalamus is the final component of the reproductive system regulating the onset of puberty in cattle and sheep (10, 14, 17). Hypothalamic concentrations of luteinizing hormone-releasing hormone (LHRH; 6) and pituitary LHRH receptors (7) do not change during sexual maturation in heifers. Therefore, the release of LHRH from the stores in the stalk median eminence appears to be the final hypothalamic component to development that precipitates the first estrous cycle. The pituitary is capable of responding to LHRH well before puberty, and treatment of heifers with estrogen

induces preovulatory surges of LH and FSH (17). Therefore, while the components for puberty are in place relatively early in life, heifers remain prepubertal until a specific sequence of endocrine changes occurs that allows estrous cycles to be initiated.

In our study, formation of a CL with a normal lifespan was induced in 114 of 203 prepubertal heifers that were either treated with P_4 alone (45 of 102 heifers) for 7 d or with P_4 for 7 d followed by an injection of EB on Day 8 (69 of 101 heifers). This outcome can potentially have an economic impact on cow/calf production because a greater proportion of heifers that are pubertal early in the breeding season can be bred at that time (5). Heifers that breed late in the breeding season wean calves that weigh less, and due to their extended postpartum anestrus period, first-calf-heifers may breed late or not breed at all in a breeding season that has a fixed number of days (22).

The hypothalamus of prepubertal heifers is more responsive to the inhibitory effects of estradiol on the release of LHRH than postpubertal heifers (7). Low prepubertal systemic concentrations of estradiol suppress the frequency that pulses of LHRH are released into the stalk median eminence. During the period preceding puberty in heifers, the number of estrogen receptors declines in the stalk median eminence (7). In addition, high doses of estradiol hasten the decline in estradiol negative feedback on LH secretion in prepubertal heifers (8), and changes in responsiveness to estradiol are a primary factor regulating the onset of puberty (14). Our data revealed that a 7-d treatment with P_4 followed by treatment with estradiol on Day 8 induced puberty in the majority of peripubertal heifers. This observation supports the mechanistic theory that administration of estradiol to peripubertal heifers reduces the negative feedback of estrogens at the hypothalamus, resulting in increased tonic release of LHRH, causing release of LH from the pituitary and thereby advancing the age at puberty.

Behavioral estrus does not always precede transient luteal phases before puberty (11), and age at puberty is not affected by removal of luteal tissue immediately after the rise in progesterone (13). However, short-term treatment with progestins induced puberty, in peripubertal heifers (18). Norgestomet is commonly used to advance the age at puberty and heifers treated with norgestomet had enhanced release of LH (1). These researchers postulated that enhanced release of LH pulses during norgestomet treatment stimulated the development of dominant ovarian follicles thus resulting in greater production of estradiol. High concentrations of estradiol in the circulation overrides the negative feedback mechanism at the hypothalamus and pituitary, and causes positive feedback that induces LHRH and LH release. This induces behavioral estrus and preovulatory surges of gonadotropins, and hence, pubertal ovulation. Our data support this theory.

Luteal function was induced in 55% of the heifers treated with progesterone. This is consistent with the notion that a progestin would reduce the negative feedback mechanism of low concentrations of estrogen on hypothalamic function in peripubertal heifers. Given the well-known effect of estradiol on inducing a surge of LH, we theorized that EB would further increase the proportion of heifers forming functional CL, as has been observed in postpartum anestrus cows (9). Our current data confirm the hypothesis that EB and progesterone may induce a preovulatory LH surge in peripubertal anestrus cattle that have insufficient endogenous estradiol production (20).

Progesterone alone (37%) or in combination with EB (81%) increased the proportion of our peripubertal heifers that exhibited signs of behavioral estrus, and 80 (P) and 94% (EB) of the heifers exhibited this induced estrus between Days 8 and 10 of the experiment (Table 3, Figure 1). Similar results were observed in postpartum anestrous beef cows using similar treatments (9).

The purpose of the current study was to evaluate the effectiveness of treating heifers with CIDRs and estrogen to induce puberty. When puberty precedes the onset of the breeding season, greater conception rates are achieved compared with when heifers are bred at the pubertal estrus (5). When heifers are managed using standard industry practices, significant percentages of heifers in herds may remain prepubertal until after initiation of the breeding season (16). It may, therefore, be an advantageous management practice to induce puberty preceding initiation of the breeding season. This should result in greater conception rates in heifers during the early portion of the breeding season. Using management practices such as those evaluated in the current study may allow for induction of puberty for purposes of preparing heifers for initiation of the breeding season.

These data suggest that the combination of short-term treatment with P₄ and an injection of EB adequately mimicked the normal endocrine mechanism for inducing estrus and normal luteal function in most peripubertal heifers. In addition, the induced estrus was synchronized within Days 2 to 4 after removal of the P₄ treatment. Sequential treatment of beef heifers with P₄ and EB can advance the age of breeding, increase calf weaning weights, and improve subsequent reproductive performance.

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