BIOLOGY OF REPRODUCTION 41, 997–1003 (1989)

Luteinizing Hormone Secretion and Corpus Luteum Function in Cows Receiving Two Levels of Progesterone[1]

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ABSTRACT

The objectives of this experiment were to determine if subnormal levels of progesterone (P_4) indicative of luteal insufficiency influence (1) pulsatile release of luteinizing hormone (LH), (2) the interval to the preovulatory surge of LH after removal of P_4, and (3) the secretion of P_4 during the estrous cycle subsequent to administration of subnormal levels of P_4. On Day 5 (Day = 0 day of estrus) of the estrous cycle, cows received P_4-releasing intravaginal devices (PRID) to produce normal (2 PRIDs; n = 7) or subnormal (0.5 PRID; n = 6) concentrations of P_4. Five cows served as controls. On Day 10, serial blood samples were collected from all cows. Collection of blood samples was again initiated on Day 17 in cows receiving PRIDs. The PRIDs were removed and blood collection continued for 78 h. Daily blood samples were collected from all animals for 42 days subsequent to estrus (estrous cycles 1 and 2, respectively). During estrous cycle 1, mean concentration of P_4 was lower ($p<0.05$) and frequency of pulses of LH was higher ($p<0.05$) in cows receiving subnormal P_4 than in cows receiving normal P_4 and control cows. Plasma concentrations of estradiol (E_2) were higher ($p<0.05$) on Days 9–16 of estrous cycle 1 in cows receiving subnormal P_4 than in cows receiving normal P_4 or in control cows. Concentrations of E_2 were greater ($p<0.05$) at 6, 18, and 30 h following removal of PRIDs in cows receiving subnormal P_4 than in cows receiving normal P_4. Onset of the preovulatory surge of LH occurred 17.1 ± 2 h earlier ($p<0.05$) in cows receiving subnormal P_4 than in cows receiving normal P_4. During estrous cycle 2, mean P_4 and duration of the luteal phase were not different ($p>0.05$) between cows in the three treatment groups. We conclude that consequences of luteal phase insufficiency on fertility may involve altered ovarian folliculogenesis modulated by associated alterations in secretion of LH.

INTRODUCTION

Defects in the functioning of the corpus luteum have been associated with infertility in cattle (Folman et al., 1973; Odde et al., 1980). Luteal phase insufficiency may be defined as a luteal phase of normal duration with reduced secretion of progesterone (P_4) by the corpus luteum of a specific estrous cycle (diZerga and Hodgen, 1981; Murdoch et al., 1983). In dairy cattle, Fonseca et al. (1983) reported conception rate at first insemination postpartum (as measured by return to estrus) increased in proportion to concentration of P_4 in circulation 12 days prior to that insemination. In addition, Erb et al. (1976) reported that mean concentrations of P_4 in plasma 48–34 h prior to the preovulatory surge of LH were higher in cows that conceived than in cows that failed to conceive. Cows that failed to conceive were observed to have a delay in the preovulatory surge of LH. Concentrations of P_4 were also higher 6 days after ovulation in cows that conceived compared to cows that failed to conceive (Erb et al., 1976).

Ovarian steroids (P_4 and 17β-estradiol [E_2]) act as modulators of secretion of LH by working independently or in concert during the bovine (Beck et al., 1976; Stumpf et al., 1988) and ovine (Karsch et al., 1980) estrous cycles. Ireland and Roche (1982) reported a negative correlation between levels of P_4 administered and secretion of LH in the bovine female. Furthermore, low levels of P_4 in the bovine female were associated with an increase in frequency of pulses of LH. Additionally, an inverse relationship between concentration of E_2 during the follicular phase and P_4 during the subsequent luteal phase of the bovine estrous cycle was reported by Rosenberg et al. (1982). Thus ovarian steroids, presumably acting through modulation of secretion of gonadotropins, may influence function of the corpus luteum during the subsequent estrous cycle.

Accepted July 25, 1989.
Received April 28, 1989.
[1]Published as Paper No. 8914 Journal Ser. Nebraska Agr. Res. Div.
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Few studies have involved administration of varying levels of P_4 to determine the influence of subnormal levels of P_4 on secretion of gonadotropins. In addition, it is not known if subnormal levels of P_4 influence other aspects of the reproductive endocrine system in cattle. Thus, the specific aims of this experiment were (1) to determine if subnormal concentrations of P_4 influence the pulsatile release of LH during the time associated with the luteal phase of the estrous cycle, (2) to compare the interval to the preovulatory surge of LH following the removal of P_4 in cows administered normal and subnormal levels of P_4, and (3) to determine if subnormal concentrations of P_4 influence function of the corpus luteum during the estrous cycle subsequent to treatment.

MATERIALS AND METHODS

Eighteen mature beef cows (2 – 4 yr of age; 458 ± 21 kg body weight) exhibiting estrous cycles at normal intervals (19 – 23 days) were used in this study. All cows were synchronized to a common day of estrus by administration of two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; UpJohn Co., Kalamazoo, MI) administered 11 days apart. On Day 5 (Day 0 = day of estrus) of the estrous cycle, cows received individual P_4-releasing intravaginal devices (PRID; CEVA, Paris, France) to produce normal (2 PRIDs; n = 7) or subnormal (0.5 PRID; n = 6) concentrations of P_4. Additional PRIDs were administered (total of 2 PRIDs) to the cows in the group receiving normal P_4 on Day 6 to avoid excessive increases in P_4 when 2 PRIDs were administered on the same day. To maintain levels of P_4, single PRIDs were removed and replaced with new PRIDs on Days 11, 12, and 16 in the cows receiving normal P_4. In the cows receiving subnormal P_4, the 0.5 PRID was removed and replaced on Day 12. The 5 remaining cows received sham devices and served as controls. In all cows receiving PRIDs, $PGF_{2\alpha}$ (25 mg) was administered on Days 6, 7, and 8 to regress the corpus luteum. Days 1 – 21 were designated estrous cycle 1 and Days 22 – 42 were designated estrous cycle 2.

On Day 10 of estrous cycle 1, catheters were inserted in the jugular vein of all cows and blood samples were collected at 15-min intervals for 24 h. Jugular catheters were again inserted on Day 17 of estrous cycle 1 in all cows that received PRIDs. Blood samples were collected at 12-min intervals for 6 h prior to removal of PRIDs. All PRIDs were then removed and blood collection continued at 12-min intervals for an additional 6 h. This was followed by blood collection at hourly intervals for 6 h. Blood was subsequently collected at 12-min intervals for 6 h. The 6-h rotation of blood collection at hourly and 12-min intervals continued up to 78 h after removal of PRIDs. All of the blood samples collected during periods of 12 min and hourly collections were allowed to clot and were stored at 4°C for 24 h. Samples were then centrifuged at 1520 × g for 15 min; serum was decanted and stored at −20°C until assayed for LH. Blood samples were collected daily in tubes treated with heparin on Days 1 – 42 from all cows. To avoid possible degradation of P_4 in blood, these samples were placed on ice immediately and plasma was separated from the blood cells by centrifugation within 2 h of collection. Plasma was then decanted and stored at −20°C until assayed for P_4. Aliquots of plasma samples from each cow on Days 1 – 4, 5 – 8, 9 – 12, and 13 – 16 were pooled to determine concentrations of E_2 during the time in which PRIDs were in place during estrous cycle 1. Similarly, aliquots of serum samples collected during the 6 h of serial blood collection on Day 17 up through the time of the preovulatory surge of LH were pooled for each cow to determine concentrations of E_2 during the follicular phase of the estrous cycle following removal of PRIDs.

Concentration of LH in all samples collected serially were analyzed by radioimmunoassay (Adams et al., 1975) using rabbit antiserum against ovine LH (TEA-RAOLH #35), highly purified ovine LH (LER-1056-C2) as radiolabeled tracer, and NIH-LH-B7 as standard. This assay has been validated in our laboratory (Wolfe et al., 1989). Intra- and interassay coefficients of variation for LH assays were 4.4% and 12.6%, respectively. Concentrations of P_4 in plasma during estrous cycles 1 and 2 were analyzed by radioimmunoassay utilizing a monoclonal antibody (Immunosearch, Emeryville, CA). Plasma samples were extracted twice with benzene:hexane (1:2), and recovery of added mass (30, 60, and 125 ng P_4) from 10 µl of serum from each of two independent serum samples averaged 105.7 ± 3.0%. Assay determinations of 10, 25, and 50 µl of sample from each of five independent serum samples were highly correlated (10 and 25 µl, r = 0.919; 10 and 50 µl, r = 0.888; and 25 and 50 µl, r = 0.974). Cross-reactivity of the P_4 antibody to corticosterone, testosterone, and E_2 were less than 0.2%. Intra- and interassay coefficients of variation for the P_4 assays were 3.8% and 11.6%, respectively. Concentrations of E_2 during the luteal and follicular phases of the estrous cycle were analyzed by radioimmunoassay (Serono Diagnostics, Norwell, MA). Serum samples were extracted twice with diethyl ether and recovery of added mass (2.5, 5.0,

TABLE 1. Mean concentrations of progesterone, area under the curve for the concentrations of progesterone, and duration of the luteal phase of cows treated with two levels of progesterone.

	Estrous cycle 1[a]		Estrous cycle 2[b]		
P_4 Treatment[c]	Mean P_4 (ng/ml)	Area[d] (units)	Mean P_4 (ng/ml)	Area[d] (units)	Luteal phase[e] (d)
Normal	6.19[g]	22.40[g]	5.39[g]	26.18[g]	14.57[g]
Subnormal	2.14[h]	7.36[h]	6.34[h]	28.68[gh]	13.50[g]
Control	6.73[g]	30.63[i]	6.61[h]	37.09[g]	16.20[g]
Pooled SEM[f]	0.29	2.30	0.35	3.20	0.87

[a]Estrous cycle 1 (Days 1–21) in which PRIDs were in place.

[b]Estrous cycle 2 (Days 22–42) following removal of all PRIDs.

[c]Treatments include normal P_4 (2 PRIDs; n = 7), subnormal P_4 (0.5 PRID; n = 6), and control (sham devices; n = 5).

[d] Area under the curve for concentrations of progesterone.

[e]Interval of time between first rise in progesterone above 1 ng/ml and decline in progesterone below 1 ng/ml of serum.

[f]Pooled standard error of mean (SEM).

[g,h,i]Numbers with different superscripts within column differ $p<0.05$.

and 10 pg E_2) from 200 µl of serum from each of three independent serum samples averaged 92.4 ± 2.6%. Assay determinations of 100 and 200 µl of sample from each of three independent serum samples were highly correlated (r = 0.999). Cross-reactivity of the antibody to E_2 for corticosterone, testosterone, and P_4 were all less than 0.01%. Intra-and interassay coefficients of variation for the E_2 assays were 3.8% and 14.6%, respectively.

Mean concentration of LH (ng/ml) in serum, frequency of pulses of LH (pulses/h), and amplitude of pulses of LH (ng/ml) were determined through the use of algorithms (Pulsar software modified for the IBM-PC by J. F. Gitzen and V. D. Ramirez, Urbana, IL). Interval to the initiation of the preovulatory surge of LH was defined as the number of hours between withdrawal of PRIDs and the initiation of a continuous high-amplitude rise in concentrations of LH. The length of the luteal phase during estrous cycle 2 was determined by calculating the number of days between the first rise in P_4 above 1 ng/ml and the decline below 1 ng/ml of plasma.

Data regarding the secretion of LH during the time in which PRIDs were in place (Day 10), interval to the preovulatory surge of LH, and concentrations of P_4 and E_2 during estrous cycles 1 and 2 were analyzed by analysis of variance (SAS, 1985), and treatment means were compared by orthogonal contrast. Secretion of LH during the follicular phase of the estrous cycle following removal of PRIDs (6-h serial samples on Day 17 through the initiation of the preovulatory surge of LH) were analyzed by analysis of variance for a repeated measures design (Gill, 1986), and treatment comparisons were determined within each 6-h period by *t*-test.

RESULTS

During estrous cycle 1, mean concentration of P_4 was lower ($p<0.05$) in cows receiving subnormal P_4 compared to cows in the control group and cows receiving normal P_4 (2.14 vs. 6.73 and 6.19 ng/ml, respectively; Table 1). Area under the curve for concentrations of P_4 during estrous cycle 1 was less ($p<.05$) in cows receiving subnormal P_4 than in cows receiving normal P_4. Area under the curve for concentrations of P_4 during estrous cycle 1 was greatest ($p<0.05$) for cows in the control group. Initiation of the follicular phase (Day 0 = day of estrus) for cows in the control group occurred on Day 18.3 ± 1 (day of decline in P_4 below 1 ng/ml). Frequency of pulses of LH (Table 2) during the time in which PRIDs were in place was higher ($p<0.05$) in cows receiving subnormal P_4 than in cows receiving normal P_4. Cows in the control group had a lower ($p<0.05$) frequency of pulses of LH than cows receiving normal P_4. Amplitude of pulses of LH (Table 2) did not differ ($p>0.05$) between cows receiving subnormal and normal P_4 and was lower ($p<0.05$) than the amplitude of pulses in cows in the control group. Mean LH (Table 2) did not differ ($p>0.05$) between cows in the control group and cows that received subnormal P_4. Mean LH was lowest ($p<0.05$) in cows receiving normal P_4. Figures 1 and 2 depict data from individual representative cows in each treatment for changes in concentrations of P_4 and pulsatile characteristics of secretion of LH, respectively, during the time in which PRIDs were in place during estrous cycle 1. Concentrations of E_2 (Fig. 3) were higher ($p<0.05$) in cows receiving subnormal P_4 on Days 9 – 12 and 13 – 16 of estrous cycle 1 compared

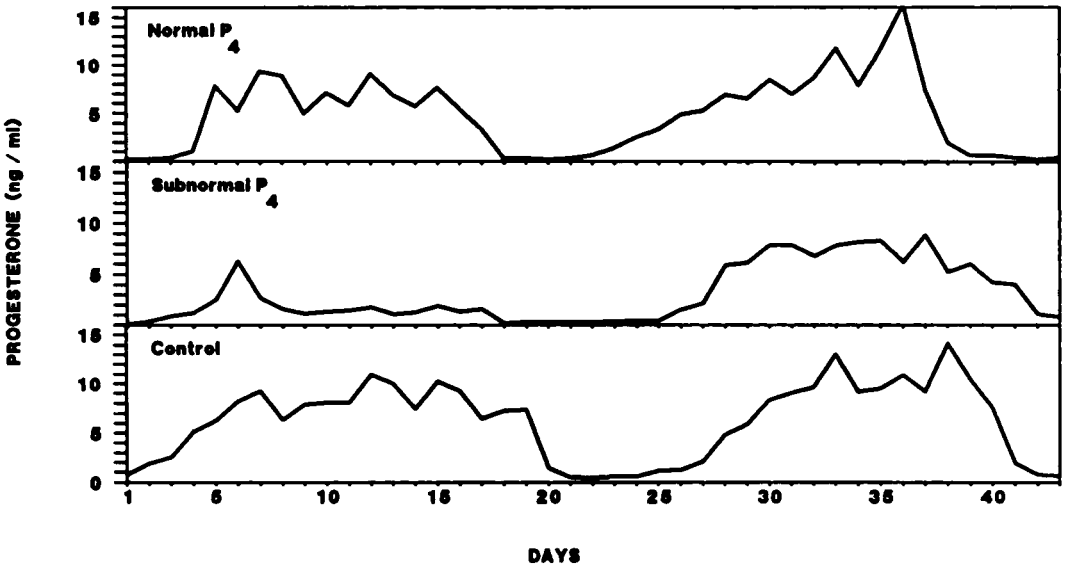

FIG. 1. Profile of secretion of progesterone for individual representative animals in each treatment group during estrous cycles 1 and 2 (Day 1–21 and Days 22–42, respectively). Treatments include normal P_4 (2 PRIDs; n = 7), subnormal P_4 (0.5 PRID; n = 6), and control (Sham devices; n = 5).

to cows in the control group and cows that received normal P_4.

During the first 6-h period immediately following the removal of PRIDs, mean concentration of LH was higher ($p<0.05$) in cows receiving subnormal P_4 than in cows receiving normal P_4. Frequency of pulses of LH was higher ($p<0.05$) in cows receiving subnormal P_4 than in cows receiving normal P_4 at 30 h following the removal of P_4. Amplitude of pulses of LH following the removal of P_4 did not differ ($p>0.05$) between cows receiving the two treatments. Concentrations of E_2 were higher ($p<0.05$) in cows that received subnormal P_4 at 6, 18, and 30 h after the removal of PRIDs compared to cows receiving normal P_4 (Fig. 4). The preovulatory surge of LH occurred 17.1 h earlier ($p<0.05$) in cows receiving subnormal P_4 than in cows receiving normal P_4 (34.3 ± 2.0 vs. 51.4 ± 2.2 h for subnormal and normal P_4 treatments, respectively). Concentrations of E_2 in cows receiving normal P_4 continued to increase up to 54 h following withdrawal of P_4 (Fig. 4). During estrous cycle 2, mean concentration of P_4, area under the curve for concentrations of P_4, and duration of the luteal phase were not different ($p>0.05$) between cows receiving the three treatments (Table 1; Fig. 1).

DISCUSSION

Patterns of secretion of LH during the luteal phase of estrous cycle 1 in the control animals in the present experiment are in agreement with those of Rahe et al., (1980) as well as Walters et al. (1984). In contrast, administration of subnormal levels of P_4 resulted in alterations of the characteristics of secretion of LH compared to animals receiving normal levels of P_4 and animals with the corpus luteum in situ. During estrous cycle 1, pulses of LH were more frequent in cows receiving subnormal levels of P_4 compared to cows receiving the two other treatments. These results are in agreement with those reported by Ireland and Roche (1982). The increase in frequency (20 to 25 pulses/24 h) of pulses of LH in cattle receiving subnormal levels of P_4 appears to be more characteristic of the pattern of secretion of LH normally observed during the follicular

TABLE 2. Mean concentrations of LH, amplitude and frequency of pulses of LH in cows treated with two levels of progesterone.

P_4 Treatment[a]	Mean LH[b] (ng/ml)	LH Amplitude[b] (ng/ml)	LH Frequency[b] (pulses/h)
Normal	1.03[d]	1.55[d]	0.61[d]
Subnormal	1.48[e]	1.34[d]	0.89[e]
Control	1.35[e]	2.32[e]	0.28[f]
Pooled SEM[c]	0.11	0.31	0.08

[a]Treatments include normal P_4 (2 PRIDs; n = 7), subnormal P_4 (0.5 PRID; n = 6), and control (sham devices; n = 5).

[b]Determined with Pulsar software.

[c]Pooled standard error of mean (SEM).

[d,e,f]Numbers with different superscript within column differ $p<0.05$.

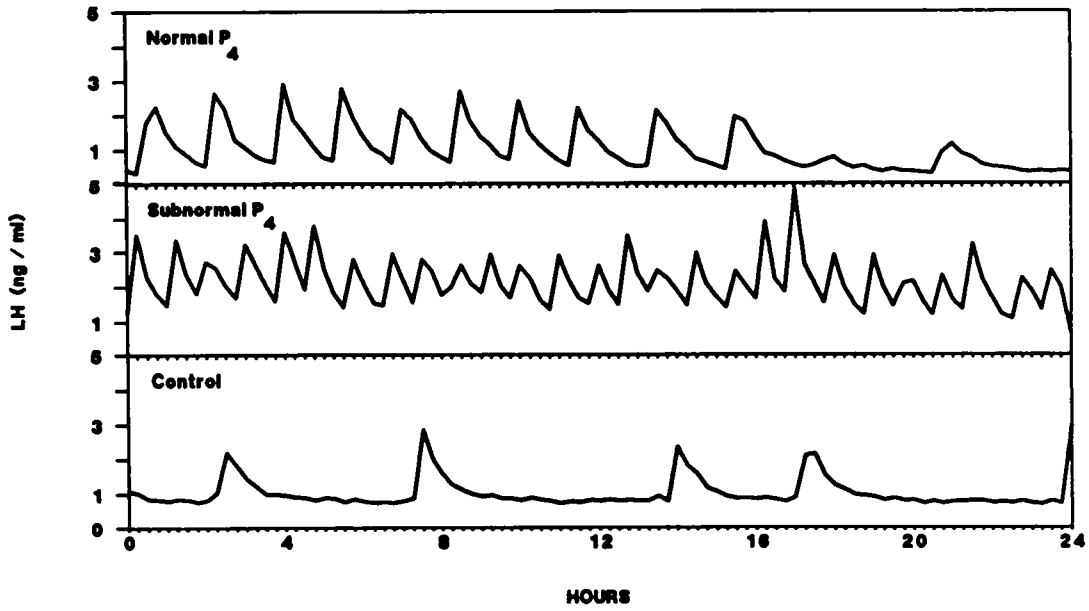

FIG. 2. Profile of secretion of LH for individual representative animals in each treatment group on Day 10 of estrous cycle 1. Treatments include normal P_4 (2 PRIDs; n = 7), subnormal P_4 (0.5 PRID; n = 6), and control (sham devices; n = 5).

phase of the estrous cycle in cows. This profile is in agreement with those observed during the follicular phases of the bovine estrous cycle by Rahe et al. (1980).

Cows treated with normal levels of P_4 exhibited mean levels of P_4 similar to those detected during the luteal phase of the estrous cycle of cows in the control group. However, cows receiving normal P_4 failed to exhibit the frequency and amplitude of pulses of LH observed in the control animals in the present study. There are several possibilities as to why the profiles of secretion of LH differed in cows in the control group and cows receiving normal concentrations of P_4. Administration of levels of P_4 attained with the normal P_4 treatment may have resulted in an inappropriate P_4:E_2 ratio modulating the hypothalamo-pituitary axis at the time of blood collection. Because ovarian steroids appear to act together to modulate gonadotropin secretion in ewes (Karsch et al., 1980) and cows (Beck et al., 1976; Stumpf et al., 1988), it seems plausible that an appropriate P_4:E_2 ratio may be necessary to achieve the pattern of secretion of LH normally observed during the luteal phase of the estrous cycle. In addition, the cows that received the normal P_4 treatment received $PGF_{2\alpha}$ to regress the corpus luteum of estrous cycle 1. The possibility exists that factors other than P_4 that are produced by the corpus luteum modulate the secretion of LH in the bovine female. From a pilot trial conducted in our laboratory prior to performing the present experiment, we determined that levels of P_4 characteristic of physiological levels could be maintained by changing PRIDs at 5-day intervals. However, during the present experiment, concentrations of P_4 were declining on Day 10 (day of blood collection while P_4 was administered) just prior to the replacement of PRIDs on Day 11 in cows receiving normal P_4. Consequently, concentration of P_4 at the time of blood collection (Day 10) was lower in cows receiving normal P_4. Concentrations of P_4 on Day 10 were 1.25 ± 0.17, 4.03 ± 0.75, and 5.91 ± 0.65 ng/ml for cows in groups receiving subnormal P_4, normal P_4, and cows in the control group, respectively. These differences in concentration of P_4 at the time of blood collection may have attributed to the differences in secretion of LH in the cows receiving normal P_4 as compared to cows in the control group.

Sirios and Fortune (1988) reported increases in ovarian follicular growth followed by apparent episodes of atresia during the normal luteal phase of the estrous cycle in cows. The most common pattern of ovarian follicle development was three waves of follicular growth that were initiated on Days 2, 9, and 16 (Day 0 = estrus) of the estrous cycle. In the present study, concentrations of E_2 in cows receiving subnormal levels of P_4 increased from Day 9 to Day 16 above that

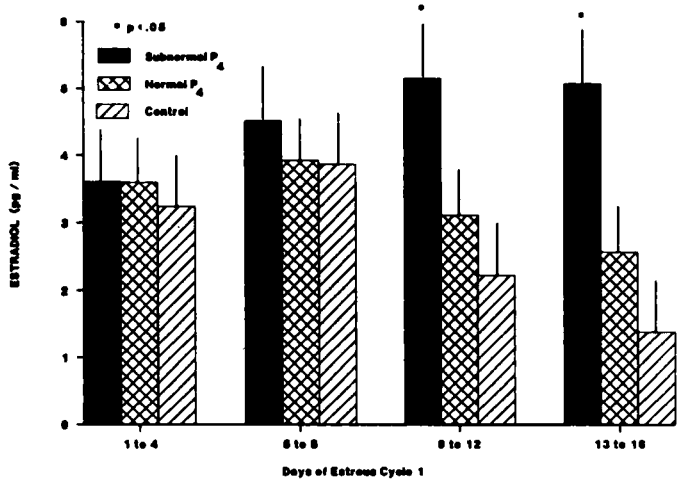

FIG. 3. Mean concentrations of 17β-estradiol from pooled plasma samples during estrous cycle 1. Treatments include normal P_4 (2 PRIDs; n = 7), subnormal P_4 (0.5 PRID; n = 6), and control (sham devices; n = 5).

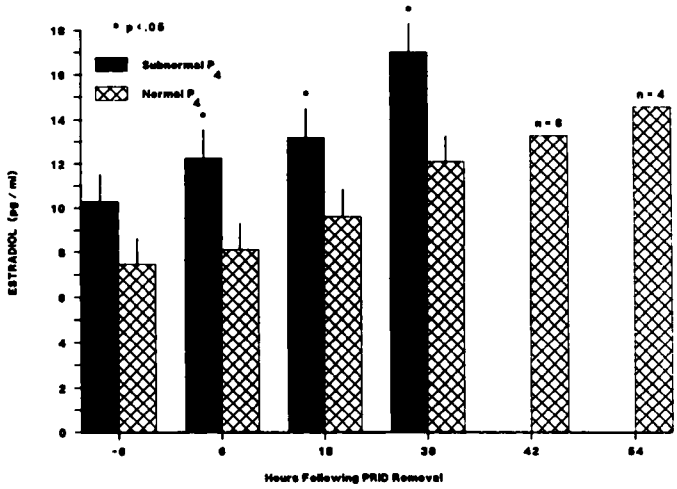

FIG. 4. Mean concentrations of 17β-estradiol during the follicular phase of the estrous cycle initiated after removal of PRIDs. Serum was pooled from samples collected serially during 6-h periods. Statistical comparisons were made up to the initiation of the preovulatory surge of LH in the cows receiving subnormal P_4 (34.3 ± 2.0 h following the withdrawal of P_4). Treatments include normal P_4 (2 PRIDs; n = 7) and subnormal P_4 (0.5 PRID; n = 6).

observed in cows in the control group and cows receiving normal P_4. This may suggest advanced maturation of ovarian follicles in cows receiving subnormal levels of P_4 during times when waves of follicular maturation appear to occur normally in cows. The increased frequency of pulses of LH on Day 10 of estrous cycle 1 in cows receiving subnormal levels of P_4 indicates that maturation of ovarian follicles during this time may be proceeding in a fashion characteristic of the follicular phase of the estrous cycle. Populations of ovarian follicles present at the initiation of the follicular phase of estrous cycle 1, following removal of PRIDs, may have been of a more advanced maturity in the cows receiving subnormal levels of P_4.

Increasing concentrations of E_2 after a decline in P_4 will induce a preovulatory surge of LH in cows (Kesner and Convey, 1982; Stumpf et al., 1987). In the present experiment, the negative feedback of P_4 on secretion of LH appeared to be lessened when concentrations of P_4 were low. Subnormal levels of P_4 did block the preovulatory surge of LH. When PRIDs were removed, the preovulatory surge of LH was initiated earlier in the cows treated with subnormal levels of P_4. Concentrations of E_2 in cows receiving subnormal levels of P_4 remained higher for the first 30 h after the withdrawal of P_4 . Interestingly, Sirios and Fortune (1988) reported that the relative size of the preovulatory follicle after the decline of P_4 was negatively correlated with the interval of time between the decline in P_4 and the preovulatory surge of LH. The possibility exists that ovarian follicles destined to ovulate subsequent to luteal phase insufficiency may be more mature. This could explain the shortened interval of time to the preovulatory surge of LH following removal of P_4 in cows receiving subnormal levels of P_4. Whether advanced maturity of ovarian follicles at the initiation of the follicular phase of the estrous cycle is detrimental to the function of the follicle is unclear. Additional research in the area of ovarian follicular dynamics and infertility during luteal phase insufficiency should be initiated. Furthermore, the influence of an altered uterine environment during luteal phase insufficiency on fertility cannot be discounted. In this case, the types of uterine secretions present during the luteal phase of a normal estrous cycle could be altered by P_4 and increased E_2.

None of the treatments imposed in the present experiment induced apparent luteal phase defects during the estrous cycle subsequent to treatment with exogenous P_4. This is in contrast to the observation of Rosenberg et al. (1982) in that changes in secretion of E_2 during the follicular phase of the estrous cycle was followed by altered function of the corpus luteum during the subsequent estrous cycle. The observed differences in secretion of E_2 during the follicular phase of the estrous cycle in the present study did not appear to be related to altered function or lifespan of the corpus luteum during the estrous cycle subsequent to treatment. The results reported by Rosenberg et al. (1982) were observed to

be dependent upon climatic conditions and parity of cow. This may explain the differences in the results of the previous and present study.

The results of the present study indicate that subnormal levels of P_4 indicative of luteal phase insufficiency alter secretion of LH and apparent ovarian activity (increased secretion of E_2) during the luteal and follicular phases of the bovine estrous cycle. Normal function of the corpus luteum occurred in the estrous cycle subsequent to treatment with subnormal levels of P_4. Therefore, the consequence of luteal phase insufficiency on fertility may involve altered ovarian processes, which are modulated by associated alterations in the secretion of gonadotropins. Subsequent studies with the animal model used in the present study will allow for detailed investigations of the association of subnormal levels of P_4 with reduced fertility.

ACKNOWLEDGMENTS

We thank Laura Rife for her patience in preparing this manuscript; Karl Moline, Bob Browleit, and Jeff Bergman for managing the experimental animals; Dr. Dan Deaver, Ken Pearson, and Georgette Caddy for technical assistance with hormone analysis; Dr. Jerry Reeves for providing LH antisera; Dr. Leo Reichert, Jr. for providing the purified LH; and The Upjohn Co. for providing the $PGF_{2\alpha}$.

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