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ABSTRACT

The objectives of this study were to determine the effects of dose of exogenous progesterone (P_4) prior to artificial insemination on concentrations of 17β-estradiol (E_2) and on conception rates in bovine females. Heifers (n = 100) and cows (n = 100) received P_4-releasing intravaginal devices (PRIDs) to produce two different circulating concentrations of P_4. All animals received a single PRID 10 days before (Day −10) the start of the breeding season (Day 0). In animals that received the low dose of P_4 (1 PRID, target concentration of 2–3 ng/ml of plasma), the original PRID remained in place for 10 days. In animals that received the larger dose of P_4 (2 PRIDs, target concentration of 5–8 ng/ml of plasma), an additional PRID was inserted on Day −9. To maintain concentrations of P_4 in the 2-PRID group, the PRIDs inserted on Days −10 and −9 were replaced with new PRIDs on Days −5 and −4, respectively. Prostaglandin $F_{2\alpha}$ (25 mg) was administered to all animals on Days −9 and −3 to remove the endogenous source of P_4. Following PRID removal, animals were artificially inseminated 12 h after signs of behavioral estrus were observed. A treatment-by-day interaction ($p < 0.0001$) was observed for concentrations of P_4 in circulation of both heifers and cows. Animals that received 2 PRIDs had greater ($p < 0.001$) concentrations of P_4 by Day −8 of treatment than animals that received 1 PRID. In cows that received 1 PRID, concentrations of E_2 increased 2.4-fold from Day −10 (6.8 ± 1.0 pg/ml) to Day −2 (16.7 ± 1.4 pg/ml). Cows treated with 2 PRIDs maintained concentrations of E_2 similar to those at the initiation of treatment (treatment-by-day, $p < 0.0001$). In contrast to cows, concentrations of E_2 did not increase in heifers treated with 1 PRID. After PRID withdrawal, both heifers and cows receiving 1 PRID exhibited behavioral estrus earlier ($p < 0.006$) than animals receiving 2 PRIDs. Cows receiving 1 PRID had a lower ($p < 0.02$) conception rate than did cows receiving 2 PRIDs (53.3% and 76.6%, respectively). Conception rate in cows was negatively correlated ($r = -0.39$; $p < 0.0001$) to mean concentration of E_2 prior to P_4 withdrawal. There were no differences in conception rates between heifers that received 1 or 2 PRIDs. In summary, cows with low circulating concentrations of P_4 (2–3 ng/ml) have elevated concentrations of E_2. Prolonged exposure to elevated concentrations of E_2 may alter the cascade of events required to establish pregnancy and thereby reduce conception rates in cattle.

INTRODUCTION

Progesterone (P_4) produced by the mammalian corpus luteum serves several important roles during the reproductive cycle in females, including regulation of secretion of gonadotropins, preparation of the uterus for implantation, and maintenance of pregnancy [1]. Deficiencies in luteal function [2, 3], either before or after mating, are associated with reduced fertility in beef [4, 5] and dairy [6–9] cattle. In cattle, concentrations of P_4 in plasma 34–48 h prior to the preovulatory surge of LH are greater in cows that conceive compared with cows that fail to conceive [10]. Therefore, the concentrations of circulating P_4 prior to estrus may influence the endocrine mechanisms required for conception.

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Administration of low doses of P_4 that produced circulating concentrations of P_4 of approximately 2 ng/ml of plasma resulted in a pattern of LH pulse frequency that more closely mimicked the follicular phase than the luteal phase patterns in cows [11–13]. Increased pulse frequency of LH during P_4 treatment was associated with continued growth and maintenance of a dominant ovarian follicle [14, 15]. As a result, concentrations of circulating 17β-estradiol (E_2) were also greater during the period of treatment with the low dose of P_4 [14, 15]. Time from P_4 withdrawal to the onset of the preovulatory surge of LH was also shortened in cows treated with a low dose (0.5 or 1 progesterone-releasing intravaginal device [PRID]) compared to cows treated with a high dose (2 PRIDs) of P_4 [11, 12]. This finding provides evidence that the dominant follicle maintained on the ovary during treatment with a low dose of P_4 is the follicle that ovulates after P_4 withdrawal.

In rats, early and/or prolonged exposure to E_2 before ovulation has been associated with decreased implantation rates, increased embryonic death, and abnormal development of embryos, including retarded growth [16–18]. Therefore, an extended period of elevated concentrations of E_2 may contribute to reduced fertility in cases of luteal deficiency prior to artificial insemination.

In cattle, our current understanding of the relationship of circulating P_4 before ovulation to fertility is based on data

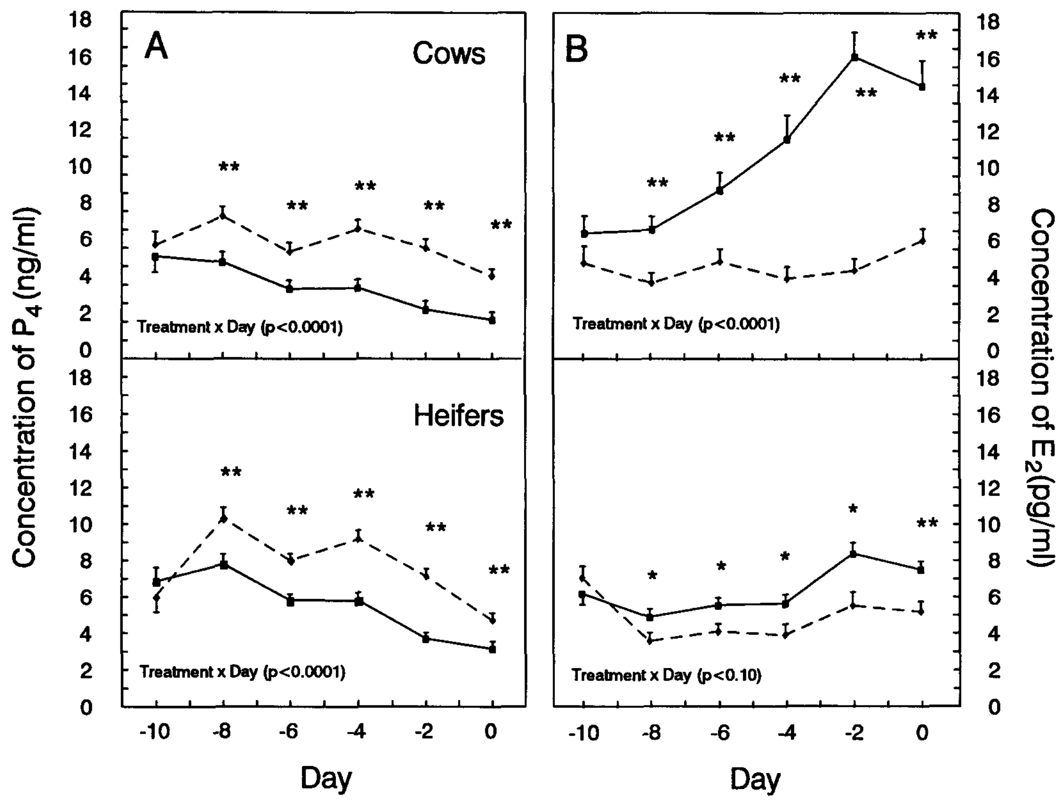

FIG. 1. Mean (± SE) concentrations of P_4 (A) and E_2 (B) in circulation of cows (n = 100) and heifers (n = 100) that received 1 (solid line) or 2 (dashed line) PRIDs during a 10-day treatment period. * $p < 0.05$; ** $p < 0.001$.

that have been examined retrospectively. Therefore, the aim of the present study was to mimic different levels of corpus luteum function by using two doses of exogenous P_4, and to examine in a prospective fashion the responses in circulating E_2 and conception rates to artificial insemination.

MATERIALS AND METHODS

Crossbred cows (47–100 days postpartum; n = 100) and heifers (13–16 mo of age; n = 100) were randomly assigned with stratification by days postpartum and age, respectively, to receive one of two doses of exogenous P_4. PRIDs (Sanofi Animal Health Inc., Overland Park, KS) were used to maintain concentrations of P_4 at two different circulating concentrations.

All animals received a single PRID 10 days before (Day −10) the start of the breeding season (Day 0). In animals that received the low dose of P_4 (1 PRID; target concentration of 2–3 ng/ml of plasma), the original PRID remained in place for 10 days. In animals that received the larger dose of P_4 (2 PRIDs; target concentration of 5–8 ng/ml of plasma), an additional PRID was inserted on Day −9. The target concentration of P_4 was based on concentrations of P_4 we detected during the midluteal phase of the estrous cycle [12]. To maintain concentrations of P_4 at 5–8 ng/ml of plasma in the 2-PRID group, the PRIDs inserted on Days −10 and −9 were replaced with new PRIDs on Days −5 and −4, respectively. All animals received prostaglandin $F_{2\alpha}$ (25 mg; The Upjohn Co., Kalamazoo, MI) on Day −9 to lyse the corpus luteum and remove the endogenous source of P_4. An additional injection of prostaglandin $F_{2\alpha}$ (25 mg) was administered on Day −3 to ensure complete luteal regression in all animals. Therefore, the primary source of circulating P_4 at the time of PRID removal (Day 0) was the exogenous source.

At the start of the breeding season (Day 0), all PRIDs were removed to simulate luteal regression. Animals were observed three times daily (0600, 1200, and 1900 h) for signs of behavioral estrus for a 7-day period with the aid of epididymally ligated bulls. Time from P_4 withdrawal to behavioral estrus was recorded for each animal. All animals were artificially inseminated 12 h after detection of behavioral estrus by one of two experienced technicians. Semen from a single ejaculation was used for all heifers and cows to decrease variation in conception rate due to differences in quality of semen. After the 7-day period of artificial insemination, all animals were exposed to fertile bulls for an additional 63 days.

Blood samples were collected every other day via jugular venipuncture starting at initiation of treatment (Day −10) through Day 46 of the breeding season. Samples were collected into heparinized tubes and placed immediately on ice to minimize degradation of P_4. Within 4 h of collection,

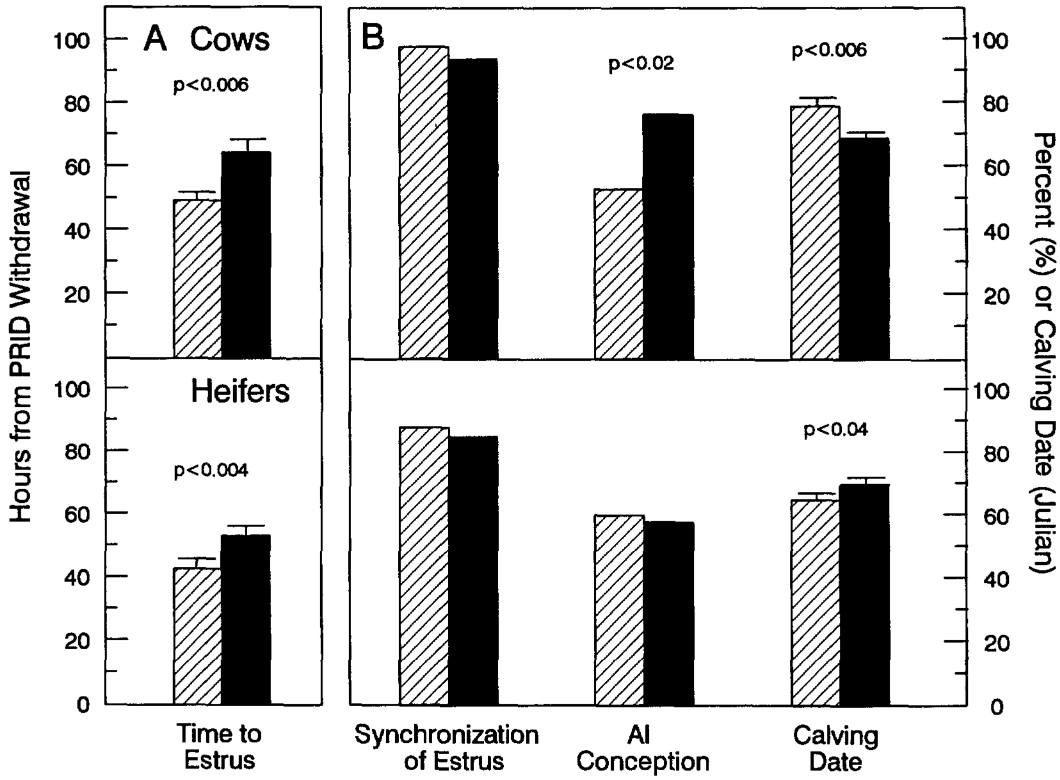

FIG. 2. A) Mean (± SE) time in hours from PRID withdrawal until observation of behavioral estrus. B) Percentages of animals observed in estrus within a 7-day period, rates of conception in response to artificial insemination, and mean Julian calving dates in cows (n = 100) and heifers (n = 100) that received 1 (hatched bars) or 2 (solid bars) PRIDs for a 10-day period prior to the start of breeding.

plasma was collected by centrifugation and stored at −20°C until concentrations of P_4 and E_2 were quantified by RIA [11, 12]. Intra- and interassay coefficients of variation for P_4 were 2.8% and 11.5%, respectively. Intra- and interassay coefficients of variation for E_2 were 2.5% and 13.2%, respectively.

Profiles of P_4 concentrations in circulation were used to determine which animals conceived as a result of artificial insemination. Animals that were inseminated and maintained concentrations of P_4 in plasma of greater than 2 ng/ml through the end of the blood sampling period (40–45 days after insemination) were considered to have conceived from artificial insemination. This was later confirmed by palpation per rectum (Days 70–80 following insemination) and subsequent calving date.

Statistical Analysis

Data were analyzed as a completely randomized design [19]. Plasma concentrations of P_4 and E_2 during treatment and after observed behavioral estrus were analyzed by analysis of variance using the general linear models procedures for repeated measures found in the SAS program [20, 21]. Time from P_4 withdrawal to behavioral estrus and calving date were analyzed by analysis of variance using the general linear models procedures of SAS. Conception rate was analyzed with Chi-square analysis using the CATMOD procedures of SAS [20].

RESULTS

Hormone Concentrations during Treatment

Cows. During the 10-day treatment, there was a treatment-by-day interaction ($p < 0.0001$) for concentrations of P_4 (Fig. 1A). Concentrations of P_4 in cows treated with 1 PRID declined from 5.6 ± 0.7 ng/ml of plasma (mean ± SE) on Day −10 to 2.2 ± 0.1 ng/ml on the day of P_4 withdrawal (Day 0). In contrast, cows that received 2 PRIDs maintained concentrations of P_4 above 5.5 ng/ml of plasma through Day −2, and concentrations of P_4 declined to 4.4 ± 0.3 ng/ml on the day of PRID withdrawal. Mean concentrations of P_4 differed ($p < 0.0001$) between the two treatment groups by Day −8.

A treatment-by-day interaction ($p < 0.0001$; Fig. 1B) was observed in concentrations of circulating E_2 in cows treated with 1 PRID compared to cows treated with 2 PRIDs. Concentrations of E_2 increased 2.4-fold from Days −10 to −2 (6.8 ± 1.0 pg/ml compared to 16.7 ± 1.4 pg/ml, respectively) in cows receiving 1 PRID and remained high until the time of P_4 withdrawal. In contrast, cows treated with 2

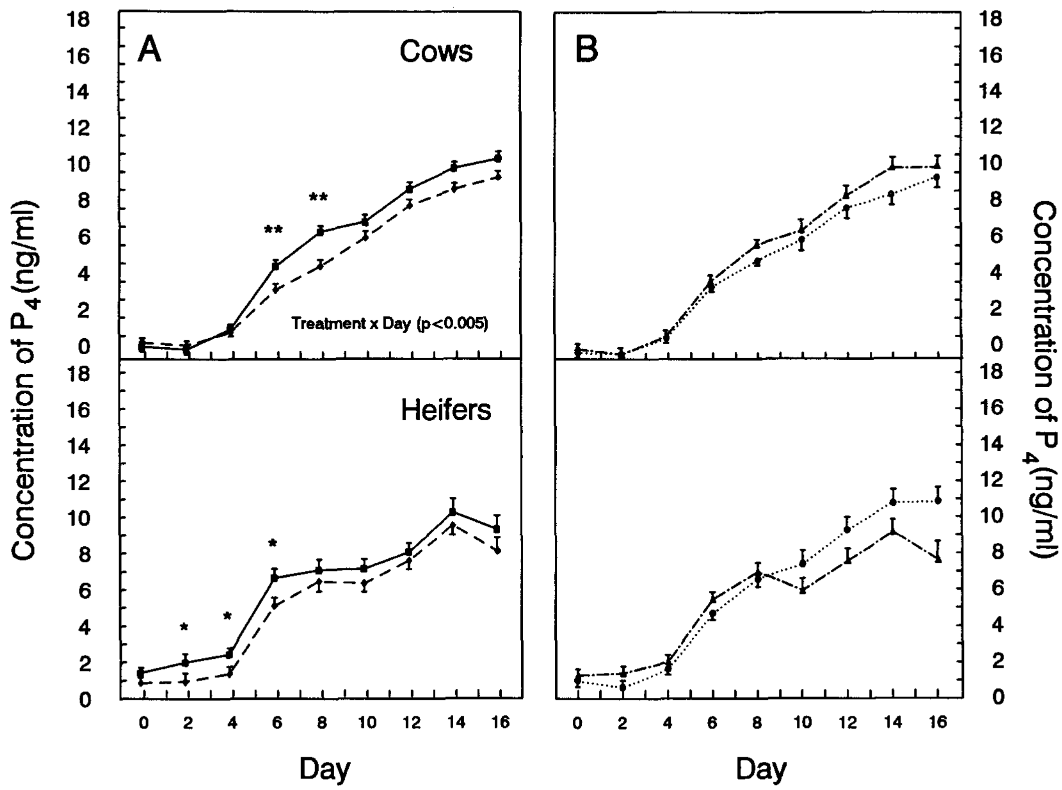

FIG. 3. A) Mean (± SE) concentrations of P_4 after observation of behavioral estrus from cows ($n = 100$) and heifers ($n = 100$) that received 1 (solid line) or 2 (dashed line) PRIDs during a 10-day treatment period. B) Mean concentrations of P_4 after observation of behavioral estrus from cows ($n = 100$) and heifers ($n = 100$) that conceived (broken line) or failed to conceive (dotted line) in response to artificial insemination independent of treatment prior to insemination. * $p < 0.05$; ** $p < 0.001$.

PRIDs had concentrations of E_2 between 4.5 and 6.0 pg/ml of plasma throughout treatment (Day −10 through Day 0).

Heifers

The exogenous doses of P_4 resulted in greater ($p < 0.0001$) concentrations of P_4 in circulation in heifers than in respective groups of cows. These differences in concentrations of P_4 were most likely due to the smaller body size of the heifers. Although absolute concentrations of P_4 were higher in heifers than in cows, the patterns of the P_4 profiles (Fig. 1A) were similar, and a treatment-by-day interaction ($p < 0.0001$) was observed. Concentrations of P_4 in heifers that received 1 PRID were maintained above 6.0 ± 0.5 ng/ml through Day −4 and declined to 3.1 ± 0.3 ng/ml at the time of P_4 withdrawal. Heifers that received 2 PRIDs had concentrations of P_4 as high as 10.5 ± 0.7 ng/ml on Day −8, and concentrations of P_4 were maintained above 7.3 ± 0.4 ng/ml until Day −2 and then declined to 4.8 ± 0.4 ng/ml at the time of P_4 withdrawal (Day 0).

Heifers in the 1-PRID group did not show an increase in concentrations of E_2 similar to that observed in cows (Fig. 1B). There was a tendency for a treatment-by-day interaction ($p < 0.10$). Concentrations of E_2 declined from Day −10 (7.1 ± 0.9 pg/ml) to Day −8 (3.7 ± 0.3 pg/ml) in heifers treated with 2 PRIDs and remained lower ($p < 0.01$) than those of heifers from the 1-PRID group through Day 0. Concentrations of E_2 in heifers treated with 1 PRID ranged from 5–8 pg/ml of plasma throughout the treatment.

Time from P_4 Withdrawal until Behavioral Estrus

Behavioral estrus occurred 10–14 h earlier ($p < 0.006$) after P_4 withdrawal in heifers and cows treated with 1 PRID (42.9 ± 2.3 and 49.6 ± 3.2 h, respectively; Fig. 2A) than in animals treated with 2 PRIDs (53.4 ± 2.7 and 64.7 ± 4.4 h, respectively; Fig. 2A). There were no differences in the percentages of animals observed in behavioral estrus within the 7-day period following P_4 withdrawal (Fig. 2B) between animals treated with 1 PRID and those treated with 2 PRIDs for heifers (88% and 85%, respectively) or cows (98% and 94%, respectively).

Conception Rate

Cows treated with 1 PRID had lower ($p < 0.02$) rates of conception in response to artificial insemination when compared to cows treated with 2 PRIDs (53.3% and 76.6%, respectively; Fig. 2B). Conception in cows was negatively correlated ($r = -0.39$; $p < 0.0001$) with mean concentra-

tion of E_2 over the 4 days prior to P_4 withdrawal. In contrast, there was no difference in conception rates in heifers (Fig. 2B) that received 1 (58.9%) or 2 PRIDs (53.9%).

When rates of conception as a result of artificial insemination and of natural service were combined, overall pregnancy rates at the end of the breeding season were not different among animals treated with 1 or 2 PRIDs in heifers (88.0% and 85.4%, respectively) and cows (86.0% and 85.7%, respectively). Mean calving date (Julian date; Fig. 2B) was earlier ($p < 0.006$) in cows treated with 2 PRIDs (69 ± 2; mean Julian date) compared to cows treated with 1 PRID (79 ± 3). In contrast, heifers treated with 1 PRID calved earlier ($p < 0.04$) than did heifers treated with 2 PRIDs (65 ± 2 and 70 ± 2, respectively).

Concentration of P_4 after Estrus

There was a treatment-by-day interaction ($p < 0.005$; Fig. 3A) for concentrations of P_4 in circulation in cows during the luteal phase following PRID withdrawal. Cows treated with 1 PRID had greater ($p < 0.001$) concentrations of P_4 by Day 6 following estrus compared to cows treated with 2 PRIDs. Increased concentrations of P_4 were maintained through Day 16 following estrus. Heifers treated with 1 PRID tended to have greater ($p < 0.10$) concentrations of P_4 during the luteal phase following PRID withdrawal compared to heifers treated with 2 PRIDs (Fig. 3A). There was no treatment-by-day interaction for the heifers. When the data were analyzed independent of treatment, there were no differences in concentrations of P_4 between animals that conceived as a result of artificial insemination and those that failed to conceive (Fig. 3B).

DISCUSSION

Results from the current study indicate that conception rates were greater in cows that received 2 PRIDs than in cows that received 1 PRID. This is the first study in which the relationship between circulating concentrations of P_4 and conception rates have been studied in a controlled experiment in cattle. Cows treated with 1 PRID had a lower rate of conception in response to artificial insemination than cows treated with 2 PRIDs. We hypothesize that the decrease in fertility associated with low concentrations of P_4, characteristic of luteal phase deficiency, was the result of prolonged exposure to elevated E_2 prior to P_4 withdrawal. In cows treated with 1 PRID, the concentrations of E_2 increased from approximately 7 pg/ml of plasma to greater than 15 pg/ml of plasma during the treatment. In contrast, heifers that received the lower dose of P_4 maintained low circulating concentrations of E_2. Concentrations of E_2 in plasma of heifers ranged from 5–8 pg/ml of plasma during the period of treatment in heifers treated with 1 PRID.

The increase in concentration of E_2 in cows that received 1 PRID in the present study may be the result of altered ovarian follicular development. Ovarian follicles that normally undergo atresia have an extended life span in cows treated with low doses of P_4 [14, 15]. Alterations in ovarian follicular development may lead to improper oocyte development, altered uterine transport of gametes and/or embryos, and/or improper preparation of the uterus for support of early embryonic development.

In the current study, cows treated with 1 PRID had increased concentrations of E_2 within 2 days after initiation of treatment. Increased concentrations of E_2 and shortened time from P_4 withdrawal to onset of behavioral estrus indicate that ovarian follicular development was altered by the lower dose of P_4. However, heifers treated with 1 PRID did not have increased concentrations of E_2. We speculate that because of the smaller body size of the heifers compared to the cows, the dose of exogenous P_4 was too great to achieve the target concentrations of P_4 in circulation (2–3 ng/ml) in heifers that received 1 PRID. Similar to cows, heifers that received 1 PRID had a shorter time from PRID withdrawal to observed behavioral estrus, but were not exposed to elevated concentrations of E_2 over an extended period of time prior to PRID withdrawal.

In previous studies, lower doses of P_4 that resulted in concentrations of circulating P_4 characteristic of luteal phase deficiency altered secretion of LH [13] and ovarian follicular activity [14, 15]. Ireland and Roche [22] reported a negative correlation between dose of progesterone administered and secretion of LH. The increase in LH pulse frequency during treatment with low doses of P_4 permitted the dominant follicle to escape normal atresia and maintain growth and dominance for a prolonged time [14, 15]. Maintenance of this dominant ovarian follicle resulted in increased concentrations of E_2 in circulation. In the current study, increased circulating concentrations of E_2 and the shortened time from PRID withdrawal to behavioral estrus were the likely results of altered ovarian follicular development. Altered ovarian follicular development and associated prolonged increased concentrations of E_2 in cows may have contributed to the reduced conception rate by a number of possible mechanisms.

One potential reason for reduced conception rates in cows with elevated concentrations of E_2 may be altered oocyte development. The role of ovarian steroids in oocyte maturation is not well understood. However, structural changes associated with oocyte maturation are associated with changes in steroid concentrations. Concentrations of P_4 and E_2 that develop in the preovulatory follicle may be involved in oocyte maturation in cattle [23]. Elevated intrafollicular concentrations of E_2 were postulated to prevent premature nuclear maturation of bovine oocytes in vivo [23]. When oocytes were examined within a few hours of removal from a follicle, less than 10% of bovine oocytes had completed meiosis I or II [24–26]. Changes associated with intrafollicular events that result in ovulation allow the oocyte to undergo nuclear maturation prior to ovulation [27].

In the present study, the prolonged exposure to concentrations of E_2 that are normally associated with the follicular phase of the estrous cycle may have compromised oocyte development in cows that received 1 PRID. This might result in ovulation of oocytes that are relatively delayed or more advanced in development. Subsequently, altered oocyte maturation may further inhibit the oocyte from successfully participating in fertilization. Therefore, one possible explanation for the greater conception rate in cows that received 2 PRIDs than in those that received 1 PRID may be altered oocyte development.

Transport of gametes in the female reproductive tract is also under the influence of ovarian steroids. Ovarian E_2 has an important role in the movement of sperm from the site of deposition to the site of fertilization. The primary mechanism of sperm transport is smooth muscle contractility and ciliary action [28]. Transport of sperm is influenced by E_2, oxytocin, and prostaglandin $F_{2\alpha}$. In the present study, prolonged exposure to E_2 may have altered transport of gametes and resulted in a decrease in fertilization rate in cows that received 1 PRID.

Impaired uterine function may also affect conception rates in animals with luteal phase deficiency. In cattle, ovarian production of E_2 and P_4 influences endometrial and myometrial function [29]. Accumulation of E_2 in endometrial cells regulates concentration of P_4 receptors in the guinea pig [30]. In turn, P_4 decreases concentrations of E_2 receptors in the myometrium [31, 32]. Maintenance of normal early embryonic development in mammals requires secretion of lipid droplets from the endometrium, which is increased by P_4 [33]. In the present study, high concentrations of E_2 prior to P_4 withdrawal may have impaired uterine preparation for maintenance of early embryonic growth. In rats, early and/or prolonged exposure to preovulatory concentrations of E_2 resulted in changes in the intrauterine environment such that it was unable to support normal embryonic development [16–18]. Prolonged exposure to elevated E_2 resulted in abnormal embryonic development, retarded growth of embryos, a decrease in implantation rate, and an increase in embryonic death in rats [16]. In the present study, the prolonged exposure to elevated concentrations of E_2 may have impaired uterine preparation for maintenance of embryonic development in cattle.

On the basis of previous and current research, we hypothesize that circulating concentrations of P_4 characteristic of luteal phase deficiency had a reduced inhibitory effect on LH pulse frequency compared to the greater concentrations of P_4 that are normally present during the midluteal phase of the bovine estrous cycle. The reduced inhibition of P_4 allows an increase in pulse frequency of LH to patterns normally occurring during the follicular phase of the estrous cycle. Increased LH support allows a dominant ovarian follicle to escape atresia and remain dominant for an extended period of time [14, 15]. The presence of a large active dominant ovarian follicle resulted in increased concentrations of E_2 for an abnormally long period of time. Prolonged exposure to elevated concentrations of E_2 or subnormal levels of P_4 may result in abnormal oocyte maturation or gamete and/or embryo transport, or in inadequate preparation of the uterus for maintenance of pregnancy. We conclude that concentrations of circulating P_4 indicative of luteal phase deficiency prior to artificial insemination reduce conception rate as a result of prolonged exposure to elevated concentrations of E_2.

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