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Pregnancy Rate Is Greater When the Corpus Luteum Is Present during the Period of Progestin Treatment to Synchronize Time of Estrus in Cows and Heifers[1]

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ABSTRACT

Our hypothesis was that conception in bovine females would be enhanced if the corpus luteum was present during the period of progestin treatment to synchronize estrus. In this study, 67 heifers (one replicate) and 124 cows (two replicates) were randomly assigned to one of two treatment groups. Seven days after estrus (Day 0), all animals were implanted with norgestomet and the implant remained in place for 10 days. All implants were removed on Day 17. Cows and heifers in one group received prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on Day 7 of the estrous cycle (PG 7; norgestomet without corpus luteum), and animals in the second group received $PGF_{2\alpha}$ on Day 17 (day of implant removal; PG 17; norgestomet with corpus luteum). All heifers and cows exhibiting behavioral estrus were artificially inseminated 12 h after estrus was detected during a 7-day period following removal of norgestomet. Blood samples were collected from cows of replicate 1 to determine serum concentrations of progesterone and 17β-estradiol. Percentage of females that had calves as a result of artificial insemination was greater ($p < 0.01$) in the PG 17 group (87% and 78% cows [two replicates] and 58% heifers) compared to the PG 7 group (31% and 44% cows [two replicates] and 41% heifers). Concentrations of 17β-estradiol in plasma were not different ($p > 0.05$; pooled SEM = 0.67) at Day 7 (beginning of the norgestomet treatment; 2.34 and 3.09 pg/ml, respectively); but they were different ($p < 0.01$) at Days 10 (9.53 and 1.65 pg/ml), 13 (12.22 and 1.31 pg/ml), and 16 (11.76 and 2.82 pg/ml for cows in the PG 7 and PG 17 groups, respectively). As we hypothesized, incidence of conception in bovine females receiving norgestomet with the corpus luteum present (PG 17) was greater than in cows treated with norgestomet in the absence of the corpus luteum. Greater concentrations of 17β-estradiol during the treatment period may contribute to the decreased incidence of conception of cows treated with the synthetic progestin in the absence of the corpus luteum.

INTRODUCTION

Increased fertility is a goal when many of the strategies used to control the reproductive system of domestic animals are incorporated into management systems. Exogenous hormones have been used to synchronize time of behavioral estrus among bovine females [1–3], but some of these treatments lead to decreased fertility. Treatment with norgestomet, a synthetic progestin, has been used as a part of the SYNCRO-MATE-B regimen (Sanofi Animal Health, Overland Park, KS) to synchronize time of estrus in cattle; however, conception resulting from insemination at the synchronized estrus is decreased [1, 3]. It has been suggested that lowered pregnancy rates after treatment with norgestomet may be due to an improper temporal relationship among estrus, the preovulatory surge of LH, and ovulation [4]. In another study, delayed selection and/or maturation of the ovulatory follicle were implicated as factors involved in reduced fertility when norgestomet was used to synchronize estrus in cows [5]. In addition, reduced fertility in cows in which time of estrus is synchronized with norgestomet may result from aberrant uterine function [6].

Effects of norgestomet on luteal life span may be mediated through effects on ovarian follicular development, which represents a key step in formation of CL with normal life span in postpartum beef cows [7]. Cows treated with norgestomet in the absence of a CL have a greater frequency of LH pulses than cows with a CL. This suggests that norgestomet does not modulate pulsatile secretion of LH in the same way as the CL [7, 8]. Increased frequency of LH pulses during norgestomet treatment increases concentrations of 17β-estradiol (E_2) compared to concentrations in untreated cows with a CL [7]. Failure of norgestomet to modulate ovarian follicular development in a manner similar to the endogenous progesterone produced by the CL may be responsible for reduced fertility when norgestomet is used to synchronize estrus. Therefore, our working hypothesis in the current study was that incidence of conception would be greater in norgestomet-treated cows in which a CL was present during the entire treatment period than in cows in which the CL was regressed at initiation of norgestomet treatment.

MATERIALS AND METHODS

Experimental Protocol

Animals used in this study were 67 heifers (1/4 Angus, 1/4 Hereford, 1/4 Red Poll, 1/4 Pinzgaur), 60 cows of replicate 1 (1/4 Angus, 1/4 Hereford, 1/4 Red Poll, 1/4 Pinzgaur), and 64 cows of replicate 2 (1/4 Hereford, 1/4 Angus, 1/4 Gelvieh, 1/4 Simmental). Females were randomly assigned to one of two treatments. All animals received the

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synthetic progestin melengestrol acetate (MGA; 0.5 mg per animal daily) in the feed for 10 days to synchronize day of estrus (Day 0 = day of estrus). Seven days after estrus, all animals were treated with implants containing 6 mg of norgestomet (17α-acetoxy-11β-methyl-19-nor-pregn-4-ene-3,20-dione). The implant was inserted in the top of the ear. Implants remained in place for 10 days to provide a continuous exogenous source of progestin. Cows and heifers assigned to be treated with norgestomet in the absence of a CL (PG 7) received prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; The Upjohn Co., Kalamazoo, MI) to regress the CL on the day of norgestomet insertion (Day 7; n = 34 heifers; n = 29 cows in replicate 1; n = 32 cows in replicate 2). Animals assigned to be treated with norgestomet in the presence of a CL (PG 17) received $PGF_{2\alpha}$ on the day of implant removal (Day 17; n = 33 heifers; n = 31 cows in replicate 1; n = 32 cows in replicate 2).

After implant removal, cows and heifers were observed for behavioral estrus three times daily (0600, 1200, and 1800 h). Sterile bulls with a section of their epididymis removed were fitted with a marking device (chinball) to assist in detection of estrus. Animals were considered to be in estrus if one or more of three criteria were met, i.e., if at least one of the following was observed: 1) a female stood for another female to mount her; 2) a female stood for the bull to mount her; 3) paint from the bull's chinball appeared on the female's back in a pattern indicating that she had been mounted by the bull. Time from norgestomet withdrawal to detection of behavioral estrus was recorded for each female. Cows and heifers were artificially inseminated 12 h after detection of estrus. To reduce variation in conception rate due to the quality of semen and differences in skills of technicians, semen from a single collection was used and only one technician inseminated cows in each replicate. After the 7-day period of artificial insemination (AI), no further breeding occurred for a 7-day period. Thereafter, cows and heifers were kept in pastures with fertile bulls for 48 days. Palpation of the reproductive tract via the rectum was performed at 60 days after the last day of AI. Conceptions to AI were recorded at this time, and date at which calves were born was used to confirm whether conception had occurred as a result of AI.

Blood samples were collected by jugular venipuncture from cows in the PG 7 and PG 17 groups of replicate 1. Samples were collected every other day from the time of implant insertion (Day 7) until removal (Day 17), and then daily through Day 45. These samples were collected in tubes with EDTA and were placed on ice immediately after collection to minimize degradation of progesterone (P_4) before samples were transported to the laboratory. Within 1 h of collection, plasma was separated by centrifugation at 2500 × *g* for 15 min and stored at −20°C until assayed for P_4 and E_2.

Concentrations of P_4 in plasma were quantified by RIA using a monoclonal antibody (BiosPacific, Emeryville, CA). This assay has been validated in our laboratory [9]. Intra- and interassay coefficients of variation were 6.6 and 10.2%, respectively. Luteal phases of less than 10 days in length, and situations in which initiation of the rise in progesterone above 1 ng/ml of plasma did not occur during the first seven days after detection of behavioral estrus, were considered to be abnormal patterns of luteal development during the estrous cycle subsequent to cessation of treatments.

Concentrations of E_2 in plasma were quantified by RIA using an antiserum to E_2 (Lilly Lot #022367 [Indianapolis, IN] at 1:2 000 000) that has been validated in our laboratory [8]. The amount of E_2 in each sample was calculated with the Four Parameter Curve Fitting Program [10]. Intra- and interassay coefficients of variation were 5.5 and 6.3%, respectively.

Statistical Analysis

Average time from implant removal to initiation of behavioral estrus was examined by ANOVA with the General Linear Models procedure (GLM) of the Statistical Analysis System (SAS) [11]. The effect of treatment on incidence of conception as a result of artificial insemination was determined by chi-square analysis with the CATMOD procedure of SAS [11]. Concentrations of progesterone and 17β-estradiol in plasma were analyzed by ANOVA using the GLM procedure for repeated measurements over time [11, 12].

RESULTS

Percentages of animals exhibiting behavioral estrus during the 7-day period of AI were not different between treatments. The pooled percentages of animals in estrus during the 7-day period of AI for the three replicates were 95 (cows, replicate 1), 91 (cows, replicate 2), and 97% (heifers). The average time from implant removal to initiation of behavioral estrus was different ($p < 0.01$) among replicates and between treatment groups, but there was no treatment-by-replicate interaction ($p > 0.10$; Table 1). The mean time from norgestomet withdrawal to initiation of behavioral estrus in females in the PG 7 group (norgestomet without CL)

TABLE 1. Time to behavioral estrus after withdrawal of a norgestomet implant in cows and heifers.

Group	Time to estrus[a,b] (h)
PG 7[c]	
Replicate 1 cow	62
Replicate 2 cows	48
Heifers	32
PG 17[d]	
Replicate 1 cow	70
Replicate 2 cows	52
Heifers	51

[a]Mean hours after removal of norgestomet implant, pooled SEM = 4.3.
[b]There was a significant effect of treatment and replicate ($p < 0.01$).
[c]Norgestomet without CL.
[d]Norgestomet with CL.

TABLE 2. Percentage of females that had calves as a result of artificial insemination, that had calves as a result of natural service, that were not pregnant after artificial insemination and natural service, or had aborted.

Treatment	Calved to artificial insemination[c] (%)	Calved to natural service (%)	Non-pregnant[d] (%)	Abortions[e] (%)
PG 7[a]				
Replicate 1 cow	28 (8/29)	48 (14/29)	14 (4/29)	10 (3/29)
Relicate 2 cows	44 (14/32)	50 (16/32)	6 (2/32)	0 (0/32)
Heifers	41 (14/34)	38 (13/34)	15 (5/34)	6 (2/34)
PG 17[b]				
Replicate 1 cow	87 (27/31)	6.5 (2/31)	6.5 (2/31)	0 (0/32)
Replicate 2 cows	78 (25/32)	19 (6/32)	3 (1/32)	0 (0/32)
Heifers	58 (19/33)	21 (7/33)	15 (5/33)	6 (2/33)

[a]Norgestomet without CL.
[b]Norgestomet with CL.
[c]There was a effect of treatment on conception rate to artificial insemination ($p > 0.01$).
[d]Not pregnant at palpation.
[e]Pregnant at palpation but did not calve.

was earlier ($p < 0.01$; 62, 48 [cows], and 32 h [heifers]) than for animals in the PG 17 group (norgestomet with CL), which showed estrus at 70, 52 (cows), and 51 h (heifers), respectively (pooled SEM = 4).

Percentages of animals in the three replicates that were pregnant by AI, pregnant by natural service, or not pregnant at the end of the study or that had aborted their conceptus are shown in Table 2. Percentage of females that were pregnant at 60 days as a result of AI differed between treatment groups ($p < 0.01$). In the PG 17 group, 75% of the females became pregnant to AI, while only 40% in the PG 7 group became pregnant. No differences in pregnancy rates were observed ($p > 0.10$) between replicates and there was no treatment-by-replicate interaction.

Average date when calves were born differed between treatment groups ($p < 0.01$, Table 3). Cows and heifers in the PG 17 group delivered their calves earlier in the calving season (76 ± 2, mean Julian date) compared to cows and heifers treated with norgestomet without the CL (84 ± 2).

Concentrations of P_4 in plasma during the treatment period were greater ($p < 0.01$) in cows in the PG 17 group than in the PG 7 group (Fig. 1). The incidence of short luteal phases (17%) and delayed CL development (7%) during the subsequent estrous cycle was greater in cows from the PG 7 than in those from the PG 17 group. In the PG 17 group, just 1 of 31 cows (3%) had a shortened luteal phase, and none of the cows had delayed development of corpus luteum subsequent to treatment. Representative data for individual cows are depicted in the profiles for animals in both treatment groups in Figure 2.

Concentrations of E_2 in plasma were not different at Day 7 (beginning of the norgestomet treatment, $p > 0.10$); however, on Days 10, 13, and 16, concentrations of E_2 were greater ($p < 0.01$) in cows in the PG 7 than in cows in the PG 17 group (Table 4).

TABLE 3. Average calving date in cows and heifers treated with norgestomet.

Group	Calving date[c,d] (Julian days)
PG 7[a]	
Replicate 1 cow	86
Replicate 2 cows	90
Heifers	78
PG 17[b]	
Replicate 1 cow	74
Replicate 2 cows	80
Heifers	74

[a]Norgestomet without CL.
[b]Norgestomet with CL.
[c]Mean ± pooled SEM = 2.
[d]There was a significant effect of treatment on calving date ($p < 0.01$).

DISCUSSION

In the present study, a greater percentage of females were pregnant as a result of AI in the PG 17 group (norgestomet

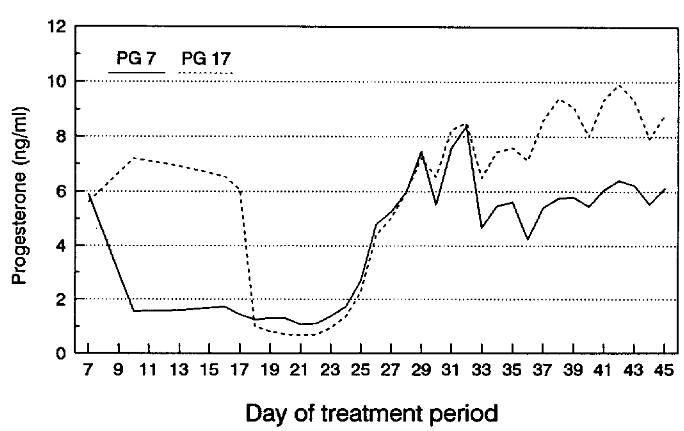

FIG. 1. Mean concentrations of progesterone (P_4) in plasma samples in PG 7 (norgestomet without corpus luteum) and PG 17 (norgestomet with corpus luteum) groups. Differences in P_4 before Day 17 were due to the treatment imposed. Differences in P_4 after Day 33 were due to differences in conception rate between the two groups; thus, corpus luteum regression occurred in a greater percentage of cows from the PG 7 group.

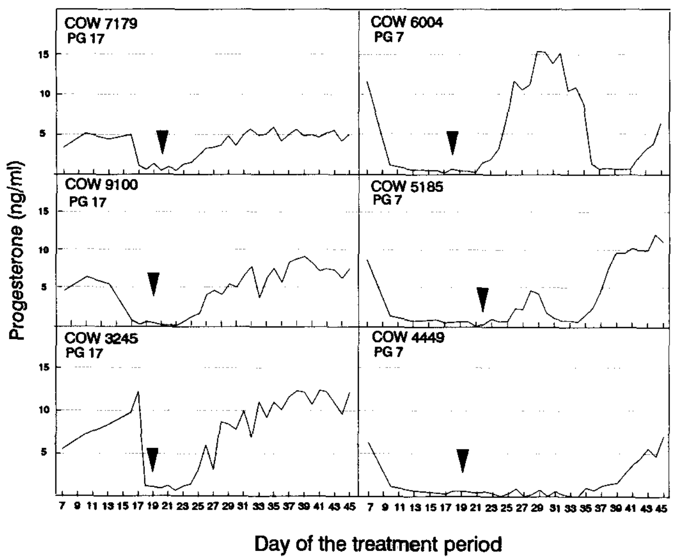

FIG. 2. Individual profiles for P_4 in cows from PG 7 (norgestomet without corpus luteum) and PG 17 (norgestomet with corpus luteum) groups. Arrow indicates time of behavioral estrus during period of artificial insemination. Note the abnormal profiles of progesterone in cows (5185 and 4449) from the PG 7 group subsequent to artificial insemination.

with CL) than in the PG 7 group (norgestomet without CL). This difference in pregnancy rate between the two treatment groups may have resulted from the greater concentrations of E_2 over a prolonged period of time in cows in the PG 7 group as compared to those in the PG 17 group. We have observed a similar increase in E_2 in cows treated with lower vs. higher doses of exogenous progesterone [8, 9, 13, 14]. Decreased pregnancy rates also occurred in cows treated with the low dose of P_4 [13]. Therefore, pregnancy appears to be compromised in cows when concentrations of E_2 are increased for periods of time longer than those occurring during the normal estrous cycle.

Intrafollicular estrogen is thought to be involved in modulation of the maturation process of bovine oocytes [15]. Exposure of oocytes to abnormally high concentrations of estrogen over extended periods of time could lead to abnormal development of oocytes in bovine females treated with norgestomet. Transport of gametes is also under control of ovarian steroids [16]. Alterations in gamete transport may result from the prolonged increase in estrogen that occurs before the time of ovulation and sperm deposition in the reproductive tract of cows treated with norgestomet. In bovine females, estrogen and P_4 influence endometrial function [17]. In the present study the synthetic progestin used to synchronize estrus and the abnormal pattern of estrogen in circulation during the treatment period may have prevented the normal development of the endometrium required for support of early embryonic development in some animals. In rats, prolonged increases in estrogen prior to ovulation result in changes in the intrauterine environment that are detrimental to early embryonic development [18, 19].

TABLE 4. Plasma concentrations of E_2 (pg/ml[a]) during the period of treatment with norgestomet.

	Day of treatment period			
Group	7	10	14	16
PG 7[b]	2.3[d]	9.6[d]	12.2[d]	11.8[d]
PG 17[c]	3.1[d]	1.7[e]	1.3[e]	2.8[e]

[a]Mean ± pooled SEM = 0.7.
[b]Norgestomet without CL.
[c]Norgestomet with CL.
[d,e]Within a column, means with different letters differ ($p < 0.05$).

Altered follicular development may be the reason for greater concentrations of estrogen in the circulation of norgestomet-treated cows without CL than in cows with a CL. Administration of low doses of exogenous P_4 results in maintenance of the dominant ovarian follicle for longer periods of time than in controls and cows administered greater doses of P_4 [14, 20]. It was found that the dominant follicles of cows treated with the low dose of P_4 failed to undergo atresia as would normally occur and produced more estrogen than did the dominant follicles of untreated control cows in the luteal phase of their estrous cycle [14, 21]. Data from a recent report indicate that cows treated with norgestomet maintain a dominant follicle for extended periods of time compared to cows with concentrations of progesterone typical of the midluteal phase of the estrous cycle [22]. Results from the present study are in agreement with those of a previous study in which norgestomet was administered to cows during diestrus and proestrus [6]. When norgestomet was administered in diestrus (in the presence of the CL), the normal wave-like pattern of ovarian follicular growth continued to occur. However, when norgestomet was administered during proestrus (in the absence of the CL), the dominant follicle was maintained for the duration of treatment and there was little development of medium or small follicles on the ovary. On the basis of these previous reports [6, 22], we propose that the dominant follicle present on Day 7 of the estrous cycle of cows in our PG 7 group was the follicle that ovulated when treatment was removed on Day 17. Cows from the PG 17 group would have ovulated from a dominant follicle that developed later in the estrous cycle, because the concentration of P_4 present in these cows would have allowed for atresia of the dominant follicle of the first follicular wave of the estrous cycle and development of other dominant follicles would have occurred [20, 22].

In the present study, intervals from cessation of treatment to time of behavioral estrus were not as accurate in determining the absolute time of initiation of estrus after treatment cessation as they would have been with more frequent estrous detection. However, comparisons between the two groups still provide useful information. The earlier onset of behavioral estrus after norgestomet removal in females in the PG 7 group as compared to those in the PG 17 group probably occurred as a result of the more advanced stage of the ovarian follicles in females in the PG 7 group at the time of treatment removal [22]. Earlier reports indicate that estrus occurs within 48 h of norgestomet withdrawal [5]. We have previously reported that the preovulatory surge of LH occurs earlier subsequent to norgestomet withdrawal than in cows that have had their CL lysed with $PGF_{2\alpha}$ [8]. These data taken together indicate that the ovarian follicles of bovine females treated with norgestomet produce a hormonal milieu that results in a more rapid cascade of behavioral (estrus) and physiological (ovulation) events after norgestomet withdrawal than after lysis of CL with $PGF_{2\alpha}$.

Aberrant follicular development may lead to development of abnormal CL because the granulosal and thecal cells of the follicle are precursors to luteal cells. In the present study there was a greater incidence of abnormal luteal development after norgestomet withdrawal in cows from the PG 7 than in those from the PG 17 group. This indicates that the endocrine and/or physiological state before ovulation led to abnormal ovarian function in some animals during the subsequent estrous cycle. This abnormal ovarian function could have contributed to the reduced incidence of conception in cows from the PG 7 as compared to those from the PG 17 group.

Previous reports indicate that the actions of norgestomet on follicular development may be mediated by an increase in frequency of LH pulses [7, 8, 22]. Increased LH pulse frequency may stimulate blood supply to a specific ovarian follicle, enhancing its development beyond that of other follicles [7]. The frequency of LH pulses in cows treated with norgestomet was similar to the pattern of LH pulses detected in cows given low doses of progesterone [8]. In a previous study, treatment with norgestomet increased the number of receptors for LH in granulosal and thecal cells, thereby increasing the sensitivity of the follicle to elevated serum concentrations of LH, which resulted in increased secretion of E_2 [23].

When treated with exogenous sources of P_4 that result in 1–2 ng/ml of plasma, heifers have greater concentrations of E_2 than do heifers administered a higher dose of P_4 that results in concentrations of 5–7 ng/ml of plasma [9]. Thus, concentration of P_4 in circulation alters ovarian activity by modulating secretion of estrogen. A higher dose of progestin than that used in the present study might suppress follicular development and estrogen secretion. Further research is being conducted in our laboratory to determine whether greater doses of synthetic progestin can mimic the CL in modulating the endogenous hormonal milieu.

In summary, when norgestomet was administered in the presence of the CL (PG 17 treatment), a greater percentage of heifers and cows were pregnant as a result of insemination at the synchronized estrus compared to norgestomet-treated animals that did not have a CL (PG 7 treatment). Norgestomet-treated animals that did not have a CL had greater concentrations of E_2 in circulation during the treatment period and had a higher incidence of luteal abnormalities during the subsequent estrous cycle than did norgestomet-treated animals that had a CL during the treatment period. We postulate that this altered endocrine milieu compromises reproduction by having a detrimental influence on the oocyte, gamete transport, and/or early embryonic development. Further research in this area will elucidate why pregnancy is compromised when synthetic progestins are administered in the doses currently used in the cattle industry to synchronize time of estrus in bovine females. More importantly, research of this nature should allow for development of procedures to synchronize estrus that allow for enhanced conception as compared those developed in the past.

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