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Abstract

The objective of this study was to monitor changes in ovarian status in heifers exposed to a progesterone insert with or without concurrent gonadotropin-releasing hormone (GnRH) injection. Estrus was manipulated in 283 heifers (31 breeding clusters) by administering GnRH, progesterone, and prostaglandin F2α(PGF2α) at 5 stages of the estrous cycle. Estrus was presynchronized with a progesterone insert for 7 days before PGF2αwas administered 24 hours before insert removal. Successive clusters of heifers were assigned to treatments (2 heifers per treatment) on cycle day 2, 5, 10, 15, and 18. Treatments consisted of a progesterone insert (day 0) for 7 days plus (1) PGF2αon day 6, 24 hours before insert removal (early PGF); (2) GnRH on day 0 + early PGF2α(GnRH + early PGF); (3) PGF2αat insert removal (late PGF); or (4) GnRH on day 0 + late PGF (GnRH + late PGF). Controls received GnRH on day 0 and PGF2αon day 7. Ovaries were scanned by transrectal ultrasonography on days 0, 2, 7, 9, and 11 to assess follicle diameters and ovulation. Blood was collected on days 0, 2, 6, 7, 8, and 9 to quantify serum concentrations of progesterone. Insemination occurred after detected estrus or by timed artificial insemination (TAI) 64 hours after insert removal. Only 25% of 141 GnRH-treated heifers ovulated by day 2; twice as many ovulated when treatment was initiated on day 5 (46.4%) than on other cycle days (20.3%). Compared with controls, progesterone concentration was greater in all progesterone-treated heifers on days 2 and 6. Early- vs. late-PGF treatment resulted in less progesterone on days 7 and 8. Pregnancy rates were less after TAI (44%) than after detected estrus (56%) and less in controls than in all progesterone treatments. Heifers in which treatments were initiated on day 10 of the cycle had the most consistent (estrus vs. TAI) pregnancy rates (65.4%) compared with heifers in which treatments were initiated on other cycle days. Compared with controls, more progesterone-treated heifers ovulated by 96 hours after insert removal. Application of the progesterone insert reduced variance of the interval to estrus after insert removal (or PGF2αinjection in controls) by 1.6-fold compared with controls. These results do not support use of GnRH in a progesterone-based synchronization protocol.; Dairy Day, 2008, Kansas State University, Manhattan, KS, 2008; Dairy Research, 2008 is known as Dairy Day, 2008

Keywords

Dairy Day, 2008; Kansas Agricultural Experiment Station contribution; no. 09-134-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 1002; Progesterone; Follicular; Estrual; Ovulation

Progesterone, Follicular, and Estrual Responses to Progesterone-Based Estrus and Ovulation Synchronization Protocols at Five Stages of the Estrous Cycle

J. S. Stevenson

Summary

The objective of this study was to monitor changes in ovarian status in heifers exposed to a progesterone insert with or without concurrent gonadotropin-releasing hormone (GnRH) injection. Estrus was manipulated in 283 heifers (31 breeding clusters) by administering GnRH, progesterone, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) at 5 stages of the estrous cycle. Estrus was presynchronized with a progesterone insert for 7 days before $PGF_{2\alpha}$ was administered 24 hours before insert removal. Successive clusters of heifers were assigned to treatments (2 heifers per treatment) on cycle day 2, 5, 10, 15, and 18. Treatments consisted of a progesterone insert (day 0) for 7 days plus (1) $PGF_{2\alpha}$ on day 6, 24 hours before insert removal (early PGF); (2) GnRH on day 0 + early $PGF_{2\alpha}$ (GnRH + early PGF); (3) $PGF_{2\alpha}$ at insert removal (late PGF); or (4) GnRH on day 0 + late PGF (GnRH + late PGF). Controls received GnRH on day 0 and $PGF_{2\alpha}$ on day 7. Ovaries were scanned by transrectal ultrasonography on days 0, 2, 7, 9, and 11 to assess follicle diameters and ovulation. Blood was collected on days 0, 2, 6, 7, 8, and 9 to quantify serum concentrations of progesterone. Insemination occurred after detected estrus or by timed artificial insemination (TAI) 64 hours after insert removal. Only 25% of 141 GnRH-treated heifers ovulated by day 2; twice as many ovulated when treatment was initiated on day 5 (46.4%) than on other cycle days (20.3%). Compared with controls, progesterone concentration was greater in all progesterone-treated heifers on days 2 and 6. Early- vs. late-PGF treatment resulted in less progesterone on days 7 and 8. Pregnancy rates were less after TAI (44%) than after detected estrus (56%) and less in controls than in all progesterone treatments. Heifers in which treatments were initiated on day 10 of the cycle had the most consistent (estrus vs. TAI) pregnancy rates (65.4%) compared with heifers in which treatments were initiated on other cycle days. Compared with controls, more progesterone-treated heifers ovulated by 96 hours after insert removal. Application of the progesterone insert reduced variance of the interval to estrus after insert removal (or $PGF_{2\alpha}$ injection in controls) by 1.6-fold compared with controls. These results do not support use of GnRH in a progesterone-based synchronization protocol.

Introduction

A timed artificial insemination (**TAI**) protocol for heifers that provides consistently acceptable pregnancy rates is lacking. Attempts to use the Ovsynch protocol as a TAI protocol for dairy heifers have proved disappointing because of poor fertility of heifers with premature expression of estrus between the first GnRH injection and $PGF_{2\alpha}$. When estrus occurs prematurely after $PGF_{2\alpha}$, a single TAI will not produce a high likelihood of conception. Most heifer developers in the beef and dairy industries desire acceptable protocols that employ TAI.

Earlier research in heifers using a progesterone-releasing intravaginal device (**PRID**), norgestomet implants, and the progesterone-releasing controlled internal drug release (**CIDR**) insert confirms the benefit of using a progestin to prevent premature expression of estrus. Expression of estrus in heifers was reported in those studies after various treatment combinations of progestins (PRID for 6 or 7 days, norgestomet for 7 days, or CIDR for 7 days, respectively) and $PGF_{2\alpha}$ given at or 24 hours before progestin withdrawal. Estrus tended to be more closely synchronized in heifers treated with $PGF_{2\alpha}$ 24 hours before progestin withdrawal than in those treated with $PGF_{2\alpha}$ concurrent with progestin removal or with $PGF_{2\alpha}$ alone. When $PGF_{2\alpha}$ was injected 24 hours before removal of the PRID or norgestomet, 76% of treated heifers were in estrus during a 24-hour period.

A recent study in beef heifers employed GnRH, progesterone (CIDR), and $PGF_{2\alpha}$ and combinations of detected estrus before AI, TAI, or both. In that 12-location study, the treatment in which GnRH was administered concurrently with a 7-day progesterone insert and TAI conducted 60 hours after insert removal and $PGF_{2\alpha}$ injection consistently produced the best pregnancy rates across locations. Necessity of the upfront GnRH injection is questionable because small differences (2.8 to 4 percentage points) in pregnancy rates were detected for heifers receiving and not receiving that injection.

The hypothesis of the current experiment was that including a progestin in a GnRH + $PGF_{2\alpha}$ protocol could prevent premature expression of estrus to facilitate TAI without loss of fertility. Variation in fertility may depend on effectiveness of the upfront GnRH injection to ovulate a dominant follicle. Further, turnover of a dominant follicle in nulliparous heifers is less successful than in lactating dairy cows, and little is known about follicle turnover or ovulatory response to GnRH in heifers treated concurrently with progesterone.

The objective of the current study was to assess follicular responses, ovulation, luteal function (concentrations of progesterone in serum), and incidences of estrus in response to combinations of GnRH, $PGF_{2\alpha}$, and progesterone applied for synchronization of estrus, ovulation, or both in nulliparous replacement heifers. An ancillary objective was to monitor pregnancy rates to determine whether a TAI protocol was feasible after using protocols consisting of GnRH, $PGF_{2\alpha}$, and progesterone.

Experimental Procedures

Holstein heifers ranging in age from 11.6 to 16.5 months (13.3 ± 0.95 months; mean ± SD) and body weight from 315 to 501 kg (410 ± 34 kg; mean ± SD) were housed at the Kansas State University Dairy Teaching and Research Center (Manhattan, KS) and maintained on dry lots with covered freestalls and a concrete feed apron. Heifers were fed a total mixed ration consisting of chopped prairie or alfalfa hay, corn or milo grain, soybean meal, and minerals and vitamins to exceed National Research Council guidelines for growing heifers.

Estrous cycles of dairy heifers (electronic estrus-detection patches were applied; HeatWatch, Cow Chips, LLC, Denver, CO) were presynchronized by placing a progesterone insert containing 1.38 g progesterone (Eazi-Breed CIDR, Pfizer Animal Health, New York, NY) for 7 days and administering 25 mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health, New York, NY) 24 hours before insert removal. After detection of estrus, heifers were assigned randomly to treatment schemes at 5 stages of the estrous cycle (days 2, 5, 10, 15, and 18).

Between February 2003 and March 2006, estrous cycles of 31 clusters of heifers (10 heifers per cluster except for 8 clusters that varied in size from 5 to 12 heifers) were presynchronized as described previously to initiate treatments in a rotating pattern starting on cycle day 2, 5, 10, 15, and 18, and then that pattern was repeated during the course of the experiment. Generally, 2 heifers per cluster were assigned randomly to each of 5 treatment schemes (Figure 1) consisting of a progesterone insert (day 0) for 7 days plus (1) 25 mg of $PGF_{2\alpha}$ (Lutalyse) on day 6, 24 hours before insert removal (early PGF); (2) 100 µg GnRH (Cystorelin, Merial, Athens, GA) on day 0 + $PGF_{2\alpha}$ on day 6 (GnRH + early PGF); (3) $PGF_{2\alpha}$ at insert removal (day 7; late PGF); or (4) 100 µg GnRH on day 0 + late $PGF_{2\alpha}$ (GnRH + late PGF) and (5) controls, which only received GnRH on day 0 and $PGF_{2\alpha}$ on day 7.

Blood was collected from a coccygeal blood vessel on days 0, 2, 6, 7, 8, and 9 (Figure 1). Blood sera concentrations of progesterone were later quantified by radioimmunoassay. Ovaries were examined by transrectal ultrasonography on days 0, 2, 7, 9, and 11 from initiation of each synchronization treatment to assess diameter of all follicles > 5 mm (days 0, 2, 6, 7, and 9), evidence for ovulation on day 2 in heifers treated with GnRH on day 0, and evidence for post-AI ovulation (day 11 or 96 hours after insert removal).

Heifers were inseminated either on the basis of standing estrus (detected by HeatWatch) or at 63.7 ± 0.8 (SD) hours (range of 61 to 65 hours) after removal of the insert. Pregnancy was diagnosed by transrectal ultrasonography at 32 or 33 days after AI. Presence of a viable embryo (heartbeat) was evidence for a confirmed pregnancy. Pregnancy rates were calculated as number of heifers pregnant after AI divided by total number of heifers inseminated.

Results and Discussion

Heifers in 2 treatments received GnRH concurrent with insertion of the progesterone insert; the control (no progesterone) received GnRH at the same time. The proportion of heifers with new ovulatory structures was evaluated 48 hours after GnRH injection. Only 25.1% of 141 heifers had new luteal structures, and a new CL was detected 48 hours after progesterone treatment in 1 heifer on cycle day 2 (Table 1). Proportions were similar among the 3 treatments in which GnRH was administered. More ($P < 0.05$) heifers ovulated on cycle day 5 than at any other stage of the cycle. No interaction was detected between stage of cycle and treatment. Concurrent administration of progesterone via the insert did not reduce subsequent ovulation because proportions of control heifers having a new luteal structure 48 hours after GnRH: day 2 (22.2%, 2/9); day 5 (55.6%, 5/9); day 10 (11.1%, 1/9); day 15 (30%, 3/10; and day 18 (27.2%, 3/11) were similar to GnRH- and progesterone-treated heifers: day 2 (21.1%, 4/19); day 5 (42.1%, 8/19); day 10 (22.2%, 4/18); day 15 (21.1%, 4/19); and day 18 (11.1%, 2/18).

Regardless of treatment, stage of estrous cycle at onset of treatment influenced largest follicle diameter on experimental day 2 because late-cycle heifers (day 15 = 9.4 ± 0.6 mm and day 18 = 8.6 ± 0.6 mm) had larger ($P < 0.05$) follicles than cycle day 10 heifers (6.1 ± 0.7 mm). By day 7, these differences were negligible, but by day 9, the largest follicle was greater ($P < 0.05$) in diameter for heifers initiating treatment on cycle days 2 (13.5 ± 0.4 mm), 10 (13.3 ± 0.4 mm), and 18 (14.6 ± 0.5 mm) than on cycle day 5 (11.7 ± 0.4 mm).

Concentrations of progesterone assessed on experimental days 0, 2, 6, 7, 8, and 9 are illustrated in Figure 2. At the onset of treatment, concentration of progesterone did not differ among heifers assigned to various treatments. By 48 hours after onset of treatment, progesterone-treated

heifers had greater ($P < 0.001$) concentrations than controls. This difference ($P < 0.05$) persisted until day 6, but among progesterone-treated heifers, those that received GnRH tended ($P = 0.08$) to have greater concentrations of progesterone than those not treated with GnRH. On day 7, 24 h after early PGF heifers were injected with PGF2α, progesterone was reduced compared with late PGF heifers. By day 8, 24 hours after insert removal, early PGF heifers tended ($P = 0.08$) to maintain lower concentrations of progesterone than late PGF heifers. By day 9, all progesterone-treated heifers had less ($P < 0.001$) progesterone than controls.

Distribution of estrus after insert removal on experimental day 7 is illustrated in Figure 3. Included in this comparison are combined treatment responses and presynchronizaton response of all heifers (pre-early PGF) in which heifers received a progesterone insert for 7 days and $PGF_{2\alpha}$ was injected 24 hours before insert removal (as in the early PGF treatment). Injection of GnRH on day 0 had no effect on onset of estrus; thus, the 2 early PGF treatments were combined as were the 2 late PGF treatments.

Among progesterone-treated heifers, distribution of estrus was shifted slightly to the left for those treated with $PGF_{2\alpha}$ 24 hours before insert removal compared with those receiving $PGF_{2\alpha}$ at insert removal. Both the pre-early PGF and early PGF (treated similar to pre-early PGF) had similar distribution patterns. Mean intervals to estrus were 44.8 ± 2.1 (pre-early PGF), 45.3 ± 3.2 (early PGF), 52.6 ± 3.3 (late PGF), and 33.4 ± 4.8 hours (control). Variances were 1,013, 829, 768, and 2,718, respectively. Variance of the first 3 groups was less ($P < 0.001$) than that of the control (Levene's test). Distribution pattern of controls was more variable because no progesterone insert was used to prevent premature expression of estrus in heifers started on treatment on cycle days 15 and 18.

Although heifers receiving $PGF_{2\alpha}$ 24 hours before insert removal were in estrus 2 to 10 hours earlier than comparable late PGF heifers, interval to estrus did not differ. Controls had a shorter ($P < 0.05$) interval to estrus than all heifers receiving progesterone inserts. Variances also differed ($P < 0.001$) among treatments and were 1.3 to 1.6 greater in control than progesterone treatments.

Post-treatment ovulation by 96 hours after insert removal is reported in Table 1. Incidence of ovulation was less ($P < 0.05$) in controls compared with progesterone treatments. Day of cycle at which treatment was initiated affected ($P = 0.001$) ovulation. Least incidence of post-treatment ovulation occurred in heifers initiating treatment on cycle day 2, and the best incidence of ovulation was detected in cycle day 10 heifers. Reduced post-treatment ovulation in control heifers was a result of premature expression of estrus and early ovulation before treatment with PGF2α and more luteolytic failures.

Pregnancy rates were recorded, but inadequate numbers of heifers were treated to detect potential differences in fertility (Table 2). Nonetheless, pregnancy rates in control heifers differed ($P < 0.05$) from those in heifers that received progesterone, and pregnancy rates after TAI were less ($P < 0.05$) than those in heifers inseminated after detected estrus. Numerically greater pregnancy rates were observed in late PGF (51.5%) than early PGF (41.3%) treatments regardless of GnRH administration. Cycle day 10 heifers had the most consistent pregnancy rates exceeding 65% regardless whether insemination occurred after detected estrus or by appointment.

One objective was to determine ovarian follicular responses to GnRH and subsequent ovulation after treatment. Injection of GnRH was rather ineffective in inducing ovulation in dairy heifers (Table 1) compared with earlier reports in heifers. Although others have suggested heifers tend to have a lesser ovulatory response to GnRH than cows because of shorter follicular waves and dominant follicles of lesser maximum diameter, a major difference in our study was the concurrent inclusion of a progesterone insert at the time of GnRH injection in all but controls. Ovulatory response to GnRH was poor and similar regardless whether GnRH administration was concurrent with progesterone. Injection of GnRH resulted in smaller follicle diameters 2 days after treatment, but compensation in rate of follicle growth produced follicles of similar size 7 days later. Injection of GnRH tended to increase serum progesterone at day 6 after onset of treatment but had no effect of interval to or duration of estrus. In contrast, GnRH-treated heifers received more mounts of greater duration during estrus. Pregnancy rates were reduced in heifers receiving TAI 64 hours after insert removal compared with those inseminated after detection of estrus. Pregnancy rates were less in controls not treated with progesterone. Administration of progesterone resulted in a more consistent and less variable pattern of estrus distribution compared with controls. Heifers initiating treatment on day 10 seemed to have the best pregnancy rates regardless whether inseminated after estrus or by appointment 64 hours after insert removal. No difference in average or variance of interval to estrus after insert removal regardless whether $PGF_{2\alpha}$ was injected at or 24 hours before insert removal justifies concurrent insert removal and $PGF_{2\alpha}$ injection. Although nonsignificant, pregnancy rates favored that management choice.

Table 1. Incidence of ovulation after gonadotropin-releasing hormone (GnRH) and post-treatment on the basis of stage of cycle at onset of treatment

	% ovulation[1] (no.)	
Item	Response to GnRH[2]	Post-treatment[2]
Treatment[2]		
Early $PGF_{2\alpha}$	2.1[a] (47)	91.5[a] (47)
GnRH + early $PGF_{2\alpha}$	28.9[b] (45)	88.9[a] (45
Late $PGF_{2\alpha}$	0.0[a] (47)	89.4[a] (47)
GnRH + late $PGF_{2\alpha}$	18.8[b] (48)	89.6[a] (48)
Control	29.2[b] (48)	68.8[b] (48)
Day of estrous cycle[3]		
2	21.4[a] (28)	75.6[ab] (45)
5	46.4[b] (28)	78.0[a] (50)
10	18.5[a] (27)	100.0[c] (45)
15	24.1[a] (29)	87.8[bc] (49)
18	17.2[a] (29)	87.0[bc] (46)

[a-c] Mean percentages within column and item having different superscript letters differ ($P < 0.05$).

[1] Determined by transrectal ultrasonographic evidence of follicle disappearance and presence of new luteal tissue 48 hours after GnRH injection or 96 hours after progesterone insert removal.

[2] All treatments except control included a 7-day progesterone insert with or without a concurrent injection of GnRH, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was given either at insert removal or 24 hours earlier. Control heifers received GnRH followed in 7 days by $PGF_{2\alpha}$.

[3] Stage of estrous cycle at onset of treatment. Excludes heifers in GnRH + early $PGF_{2\alpha}$ and GnRH + later $PGF_{2\alpha}$ heifers that did not receive a GnRH injection.

Table 2. Pregnancy rates in response to treatment with gonadotropin-releasing hormone (GnRH), progesterone, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) after detected estrus or timed artificial insemination (TAI)[1]

	% Pregnant (no.)		
Item	Estrus	TAI	Total
Treatment[2]			
Early $PGF_{2\alpha}$	53.3 (15)	40.0 (40)	43.6[x] (55)
GnRH + Early $PGF_{2\alpha}$	50.0 (16)	42.5 (40)	44.6[x] (56)
Late $PGF_{2\alpha}$	42.9 (7)	54.2 (48)	52.7[x] (55)
GnRH + Late $PGF_{2\alpha}$	63.6 (11)	49.0 (49)	51.7[x] (60)
Control	75.0 (8)	30.4 (46)	37.0[y] (54)
Total	56.1[a] (57)	44.0[b] (223)	46.4 (280)
Early $PGF_{2\alpha}$	51.6 (31)	41.3 (80)	44.1 (111)
Late $PGF_{2\alpha}$	55.6 (18)	51.5 (97)	52.2 (115)
Day of estrous cycle[3]			
2	66.7 (6)	34.7 (49)	38.2 (55)
5	33.3 (3)	37.9 (58)	37.7 (61)
10	66.7 (9)	65.2 (46)	65.4 (55)
15	62.5 (16)	42.5 (40)	48.2 (56)
18	47.8 (23)	36.7 (30)	41.5 (53)

[a-b] Mean percentages within row having different superscript letters differ ($P < 0.05$).

[x-y] Mean percentages within column having different superscript letters differ ($P < 0.05$).

[1] Pregnancy rates determined by transrectal ultrasonographic evidence of fluid, embryonic heart beat, and presence of a corpus luteum at 32 to 33 days post-TAI.

[2] All treatments except control included a 7-day insert with or without a concurrent injection of GnRH, and $PGF_{2\alpha}$ was given at either insert removal or 24 hours earlier. Control heifers received GnRH followed in 7 days by $PGF_{2\alpha}$.

[3] Stage of estrous cycle at onset of treatment.

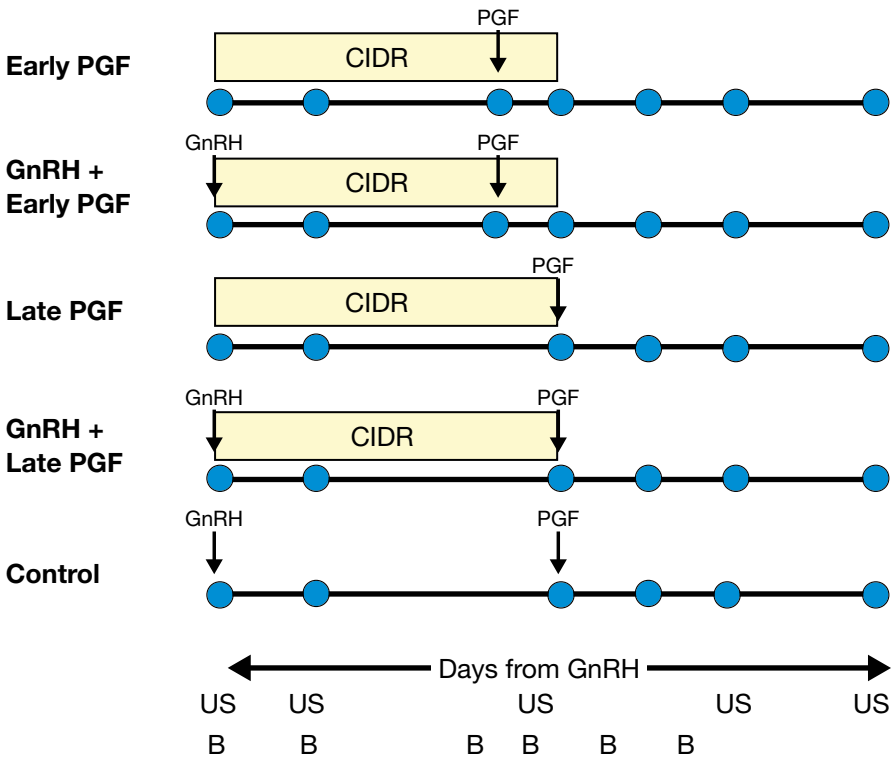

Figure 1. Experimental design of treatments. All treatments except control included a 7-day progesterone insert with or without a concurrent injection of GnRH. An injection of $PGF_{2\alpha}$ (PGF) was given either at insert removal or 24 hours earlier. Control heifers received GnRH followed in 7 days by $PGF_{2\alpha}$. CIDR = 1.38 g of progesterone controlled internal drug release insert; GnRH = 100 µg of GnRH; PGF = 25 mg of $PGF_{2\alpha}$; US = transrectal ultrasonography; and B = blood collection.

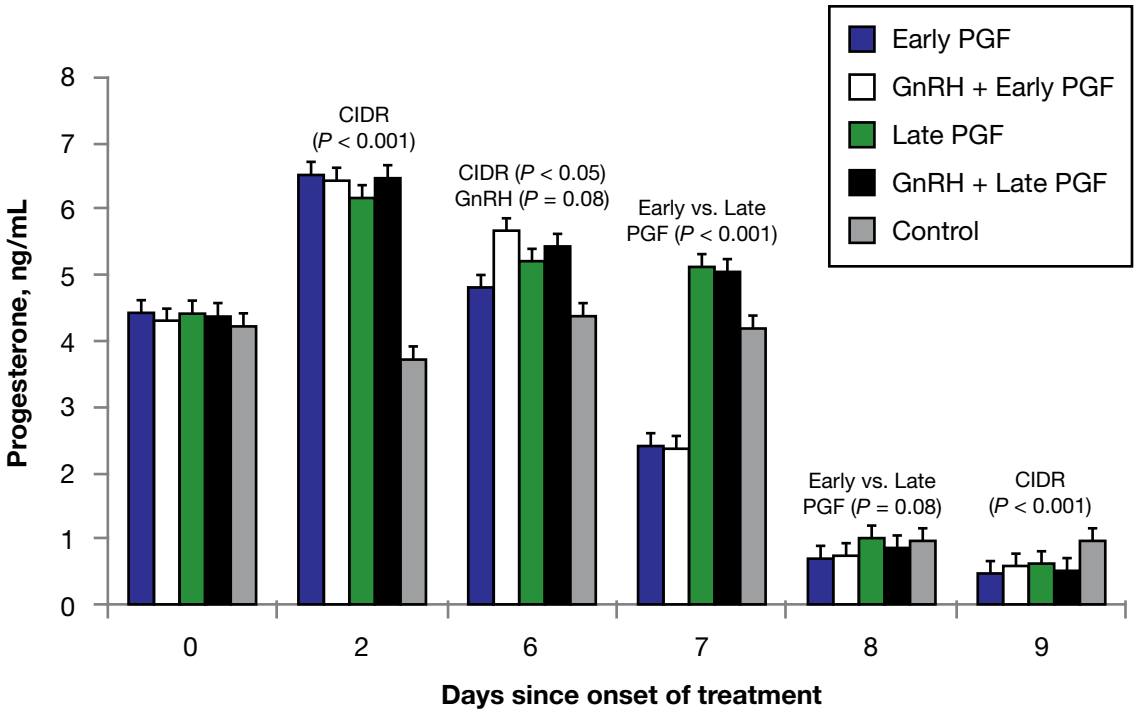

Figure 2. Treatment effects on concentrations of progesterone beginning at the onset of treatment (day 0) until 2 days after progesterone insert removal. All treatments except control included a 7-day insert with or without a concurrent injection of GnRH. An injection of $PGF_{2\alpha}$ (PGF) was given either at insert removal or 24 hours earlier. Control heifers received GnRH followed in 7 days by $PGF_{2\alpha}$. Five treatments were (1) early PGF (n = 56), (2) GnRH + early PGF (n = 56), (3) late PGF (n = 55), (4) GnRH + late PGF (n = 60), and (5) control (n = 55). Contrasts are progesterone insert (CIDR) vs. control, GnRH vs. no GnRH (progesterone insert treatments only), and early vs. late PGF.

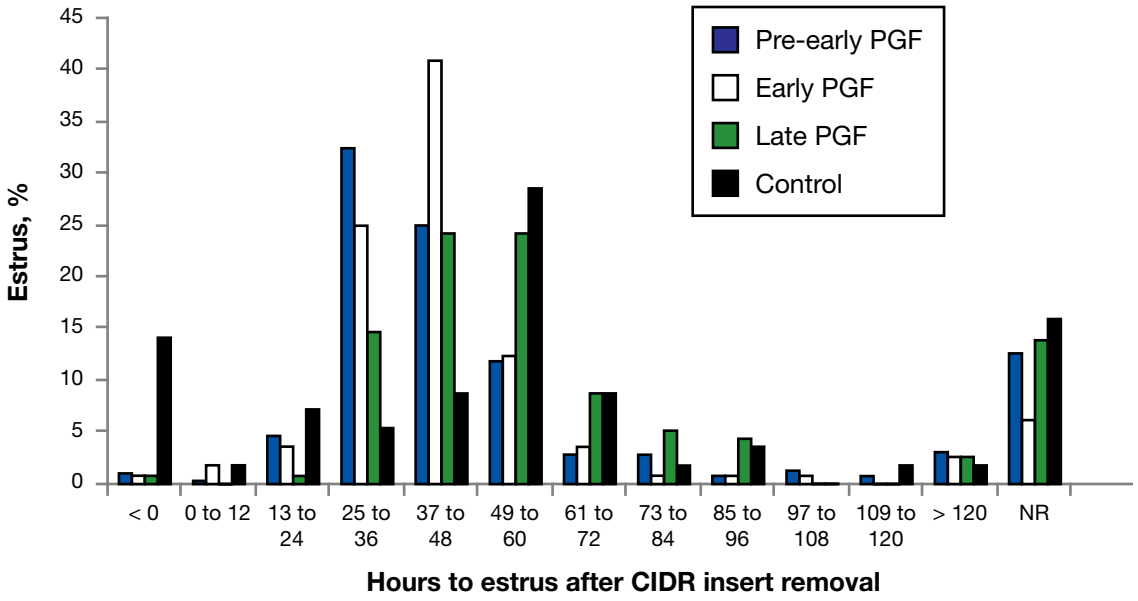

Figure 3. Pattern of post-treatment estrus. Distribution of estrus after either early administration of $PGF_{2\alpha}$ (PGF; 24 hours before a 7-day progesterone insert was removed from all heifers during pretreatment synchronization of estrus; n = 247), early $PGF_{2\alpha}$ (24 hours before insert removal) during treatment (n = 105), late $PGF_{2\alpha}$ concurrent with insert removal (n = 99), or in controls (GnRH 7 days before $PGF_{2\alpha}$; n = 47).