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Altered progesterone concentrations by hormonal manipulations before a fixed-time artificial insemination CO-Synch + CIDR program in suckled beef cows

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ABSTRACT

We hypothesized that pregnancy outcomes may be improved by inducing luteal regression, ovulation, or both (i.e., altering progesterone status) before initiating a timed AI program in suckled beef cows. This hypothesis was tested in two experiments in which cows were treated with either $PGF_{2\alpha}$ (PG) or PG + GnRH before initiating a timed AI program to increase the proportion of cows starting the program in a theoretical marginal (< 1 ng/mL; Experiment 1) or elevated (≥ 1 ng/mL; Experiment 2) progesterone environment, respectively. The control was a standard CO-Synch + CIDR program employed in suckled beef cows (100 μg GnRH im [GnRH-1] and insertion of a progesterone-impregnated intravaginal controlled internal drug release [CIDR] insert on

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study Day -10, 25 mg PG im and CIDR insert removal on study Day -3, and 100 μg GnRH im [GnRH-2] and timed AI [TAI] on study Day 0). In both experiments, blood was collected before each injection for later progesterone analyses. In Experiment 1, cows at 9 locations (n = 1,537) were assigned to either: (1) control or (2) PrePG (same as control with a PG injection on study Day –13). The PrePG cows had larger ($P < 0.05$) follicles on study Day –10 and more ($P < 0.05$) ovulated after GnRH-1 compared with control cows (60.6 vs. 36.5%), but pregnancy per TAI was not altered (55.5 vs. 52.2%, respectively). In Experiment 2, cows (n = 803) at 4 locations were assigned to: (1) control or (2) PrePGG (same as control with PG injection on study Day –20 and GnRH injection on study Day –17). Although pregnancy per TAI did not differ between control and PrePGG cows (44.0 vs. 44.4%, respectively), cows with BCS > 5.0 or ≥ 77 d postpartum at TAI were more ($P < 0.05$) likely to become pregnant than thinner cows or those with fewer days postpartum. Presynchronized cows in both experiments were more ($P < 0.05$) likely than controls to have luteolysis after initial PG injections and reduced ($P < 0.05$) serum progesterone; moreover, treatments altered the proportion of cows and pregnancy per TAI of cows in various progesterone categories before the onset of the TAI protocol. In combined data from both experiments, cows classified as anestrous before the study but with elevated progesterone on D –10 had increased ($P < 0.05$) pregnancy outcomes compared with anestrous cows with low progesterone concentrations. Progesterone concentration had no effect on pregnancy outcome of cycling cows. In summary, luteal regression and ovulation were enhanced and progesterone concentrations were altered by presynchronization treatments before the 7-d CO-Synch + CIDR program, but pregnancy per TAI was not improved.

1. Introduction

Widespread acceptance of AI in beef cattle partly depends on the success of programs that facilitate insemination at a predetermined time. Several ovulation synchronization protocols that use exogenous GnRH at the time of insertion of a progesterone-impregnated intravaginal controlled internal drug release (CIDR) insert have been developed [1,2]. Beef herds that have >50% anestrous cows at the start of the breeding season may benefit from protocols that promote ovulation in response to GnRH before initiating a timed AI (TAI) program. Dominant follicles are present in noncycling cows [3], and exogenous GnRH-induced ovulation of a dominant follicle [4,5].

Efficacy of GnRH-induced LH secretion resulting in ovulation of ovarian follicles depended on maturity of the follicle exposed to GnRH [6]. Follicles induced to ovulate before reaching 11 mm in diameter were less likely to result in pregnancy than ovulation of larger follicles in beef cows [7]. Replacement beef heifers were more likely to become pregnant when follicles induced to ovulate with GnRH ranged from 10.7 to 15.7 mm in diameter [8]. In dairy cows, follicles > 10 mm in diameter ovulated in response to GnRH [9]; however, only 66% of beef cows ovulated after a single injection of GnRH because of variation in follicle size [10].

Using $PGF_{2\alpha}$ (PG) to synchronize a follicular wave before the start of a 6-d CO-Synch TAI protocol improved pregnancy outcomes in beef cows compared with a 5-d CO-Synch (GnRH injection 5 d before and 66 to 70 h after PG, with GnRH-2 injection given at TAI) + CIDR insertion concurrent with the GnRH-1 injection [11]. Likewise, a larger proportion of heifers exhibited a new follicular wave at the start of a TAI protocol when they were injected with PG 3 d before the initiation of the protocol [12]. Lactating dairy

cows had better pregnancy outcomes during the summer when they were treated with PG and GnRH (3 d after PG and 7 d before the start of the Ovsynch protocol) than cows whose estrous cycles were presynchronized with two PG injections 14 d apart, with the second PG injection administered 10 d before Ovsynch [13].

We hypothesized that treatment with PG or PG followed by GnRH before the start of the 7-d CO-Synch + CIDR protocol would improve pregnancy outcomes by altering concentrations of progesterone at the onset of the TAI protocol. Our objectives were to determine if presynchronization treatments would increase the proportion of cows beginning a 7-d CO-Synch + CIDR protocol either at a low (Experiment 1) or high (Experiment 2) progesterone environment and consequently improve pregnancy outcomes compared with the 7-d CO-Synch + CIDR control.

2. Materials and methods

2.1. Experiment 1: Experimental design

A total of 1,537 primiparous and multiparous cows at nine locations in four states (Florida, Georgia, Kansas, and South Dakota, USA) were enrolled. Characteristics of suckled beef cows enrolled by location are summarized in Table 1. Cows were stratified by breed, days postpartum, and parity and assigned randomly to two treatments (Fig. 1). Control cows received the standard CO-Synch + CIDR program (100 μg GnRH [2 mL Factrel, Pfizer Animal Health, Whitehouse Station, NJ] 7 d before and 72 h after 25 mg PG [5 mL Lutalyse; Pfizer Animal Health]). A new CIDR insert (Pfizer Animal Health) containing 1.38 g progesterone was placed intravaginally at the time of the first GnRH

injection (study Day –10; d 0 = TAI). Experimental cows (PrePG) received 25 mg PG 3 d before (study Day –13) the CO-Synch + CIDR program began. The PrePG treatment was designed to create a larger proportion of cows with marginal concentrations of progesterone (< 1 ng/mL) at the onset of the TAI protocol.

Body condition scores (1 = thin; 9 = very fat) were assigned on study Day –13 and estrus-detection patches (Estrotect, Rockway, Inc. Spring Valley, WI) were affixed to all cows at each location (except GA). Cows with missing patches were not included in the analysis. Estrus-detection patches were removed on study Day –10 and scored (0 = not colored, 1 = partially colored, and 2 = completely colored). On study Day –3, CIDR inserts were removed, a second estrus-detection patch was applied, and $PGF_{2\alpha}$ was administered to all cows in both treatments. Only 3.6% of patches were scored as 1 (partially colored) by study Day –10 (3 d after the treatment PG injection), and 5.5% of patches were scored as 1 at timed AI; therefore, we eliminated patch score information from cows with patch scores of 1 and assumed that cows with patch scores of 0 did not show estrus. We further assumed that cows with completely colored patches (scored as 2) had been mounted and were in estrus sometime after either PG injection.

Artificial insemination was performed 72 h after CIDR insert removal on study Day 0 and estrus-detection patches were removed and scored. Cows were either exposed to cleanup bulls beginning 10 to 12 d after TAI or re-inseminated at subsequent estrus (KS-P location; Table 1). At 35 d after TAI, pregnancy was confirmed by transrectal ultrasonography (Aloka 500V, 5 MHz transrectal transducer, Wallingford, CT). A positive pregnancy outcome required the presence of a corpus luteum (CL) and uterine fluid or an embryo with a heartbeat. A final pregnancy diagnosis was determined via

transrectal ultrasonography at least 35 d after the end of the breeding season (removal of natural service sires or termination of detection of estrus and AI). Embryonic losses in cows that conceived to the TAI were then determined.

2.2. Experiment 1: Cycling status

Blood samples were collected via caudal vessel puncture from cows at seven of the nine locations (except FL-1 and FL-2; Table 1) at study Days –23, –13, –10, –3, and 0 and later assayed for progesterone concentration. Serum progesterone concentration was measured in all blood samples by direct quantitative (nonextracted) radioimmunoassay using Coat-A-Count progesterone kits (catalog # TKPG; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) previously validated for bovine serum [14]. Intra- and interassay CVs for progesterone were 6.5 and 9.3%, respectively. Assay sensitivity was 7.8 pg/mL.

Progesterone concentrations were categorized as high (≥ 1 ng/mL unless a CIDR was in situ, then ≥ 2 ng/mL) or low (< 1 ng/mL). Blood samples collected on study Day –23 and –13 were used to determine cyclicity. Cows with high progesterone on study Day –23 or –13 were defined as cycling. Additional cows that had no blood samples but had a fully colored patch (score = 2) were deemed to be cycling on study Day –13. Any cow that had low progesterone status on study Day –23 and –13 was defined as anestrous. The sample collected at study Day –3 reflected progesterone concentrations resulting from the CIDR insert, a functional CL, or both.

2.3. Experiment 1: LH concentrations

Four additional blood samplings were performed via caudal vessel puncture on a subset of cows (n = 62) at one location (KS-P) at 0 and 2 h after GnRH injections on study Day –10 and 0. Serum from these two samples was analyzed for concentrations of LH by liquid-phase double-antibody radioimmunoassay [15]. Sera were assayed in triplicate (100 μL) in one assay with an intra-assay CV of 2.95% for a bovine serum pool that averaged 10 ± 0.3 ng/mL. Pooled sera assayed in quadruplicate at 25, 40, 60, 100, 175, 200, and 300 μL averaged 9.72 ng/mL and paralleled the standard curve.

2.4. Experiment 1: Ovarian structures

Ovaries in a subset of cows (n = 188) at two locations (GA and KS-P; Table 1) were scanned by transrectal ultrasonography at study Day –10 and –3. Follicles ≥ 6 mm in diameter were measured using the internal calipers of the ultrasound machine. Follicle diameter was determined by averaging the diameter of the follicle at its widest point and the diameter at a right angle to the first measurement. Presence of all luteal structures was recorded on both days, and new luteal structures observed on study Day –3 were noted.

2.5. Experiment 1: Statistical analyses

Concentrations of progesterone on all sampling days were analyzed by ANOVA using a general linear model (procedure GLM, SAS Institute Inc., Cary, NC, USA). The model included treatment (n = 2), location (n = 6), parity (primiparous vs. multiparous), BCS (≤ 5 vs. > 5), all 2-way interactions with treatment, and median days postpartum (< 75 vs. ≥ 75 d) at TAI. Treatment differences were detected by F-test.

Serum concentrations of LH for a subset of cows collected at 0 and 2 h after each GnRH injection on study Day –10 and –3 were analyzed using a mixed linear model (procedure MIXED, SAS Institute Inc.). The model included treatment, time, cycling status, BCS (≤ 5 vs. > 5), median days postpartum, and all two- and three way-interactions of cycling, treatment, and time.

Binomial fertility responses were analyzed separately for pregnancy per TAI (35 d post-TAI), final pregnancy rate at the end of the breeding season, and subsequent pregnancy loss using the GLIMMIX procedure (SAS Institute Inc.), with location as a random effect and treatment, parity, BCS (≤ 5 vs. > 5), median days postpartum, and all two-way interactions with treatment as fixed effects.

Cows with high (≥ 1 ng/mL) progesterone at the time of either PG injection and then low progesterone at the following blood collection (3 d later) were assumed to have had luteolysis. Treatment effects on estrus and luteolysis measures were analyzed using the GLIMMIX procedure, with location as a random effect and parity, BCS (≤ 5 vs. > 5), median days postpartum, and two-way interactions of treatment with parity and BCS as fixed effects.

Pregnancy responses were examined when cows were grouped into selected progesterone concentration categories at the time of CIDR insertion (study Day –10). Four progesterone categories examined were low (< 0.5 ng/mL), low-medium (0.5 to

1.99 ng/mL), high-medium (2.0 to 3.99 ng/mL), and high (≥4.0 ng/mL). A model including treatment, herd, median days postpartum, parity, BCS (≤ 5 vs. > 5), and progesterone categories was analyzed using the GLIMMIX procedure to determine odds ratios of pregnancy and proportions of cows within each progesterone category.

The largest follicle diameter was measured by transrectal ultrasonography at two different times (study Days –10 and –3) in cows at two locations (GA and KS-P). Measurements were analyzed by a general linear model (procedure GLM). The statistical model included treatment, location, parity, breed (n = 6), BCS (≤ 5 vs. > 5), cyclicity, and median days postpartum as fixed effects. Two-way interactions between treatment and location, parity, BCS, and cyclicity also were examined. Variance between the largest follicle diameters on study Days –10 and –3 between treatment and control cows were compared using Levene's test [16], assuming that less variance of follicle diameters represented improved follicular synchrony.

2.6. Experiment 2: Experimental design

A total of 803 primiparous and multiparous cows at four locations in two states (Florida and Kansas) were utilized. Characteristics of suckled beef cows enrolled by location are summarized in Table 1. Cows were stratified by breed, days postpartum, and parity, and then assigned randomly to two treatments (Fig. 1). Day 0 was the day of timed AI. Control cows received the same CO-Synch + CIDR protocol as defined in Experiment 1. Treated cows (PrePGG) received 25 mg PG (study Day –20) followed by 2 mL Factrel 7 d before the CO-Synch + CIDR program was initiated. The PrePGG

treatment was designed to create a larger proportion of cows with elevated concentrations of progesterone (≥ 1 ng/mL) at the onset of the TAI protocol.

2.7. Experiment 2: Cycling status

Blood samples were collected via caudal vessel puncture on study Day −20, −17, −10, −3, and 0 and later assayed for progesterone concentration. Serum progesterone concentration was measured by radioimmunoassay. Intra- and interassay CVs for progesterone were 3.4 and 7.6%, respectively. Assay sensitivity was 1.9 pg/mL. As in Experiment 1, progesterone concentrations were categorized as either high (≥ 1 ng/mL) or low (< 1 ng/mL). Blood samples from study Day −20, −17, and −10 were used to determine cyclicity. Cows with high progesterone on any sampling day (study Day −20, −17, or −10) were defined as cycling. Cows with low progesterone on all three sampling days were defined to be anestrous. The sample collected on study Day −3 reflected progesterone concentrations resulting from the CIDR insert, a functional CL, or both.

Body condition scores (1 = thin; 9 = very fat) were assigned to all cows at the time PG was administered to the experimental group on study Day −20. Artificial insemination was performed 60 to 72 h after CIDR insert removal on study Day 0. Cows were either exposed to cleanup bulls beginning 10 to 12 d later or reinseminated at subsequent estrus (KS-P location; Table 1). Pregnancy at 35 d after TAI, pregnancy at least 35 d after the end of the breeding season, and embryo loss were determined by transrectal ultrasonography.

2.8. Experiment 2: Ovarian structures

Ovaries of a subset of cows (n = 169) at one location (FL; Table 1) were scanned using transrectal ultrasonography on study Day −17, −10, and −3. Follicles ≥ 6 mm in diameter and all luteal structures were measured using the internal calipers of the ultrasound machine as described in Experiment 1. Volume of each luteal structure was calculated [$4/3 \times r^3 \times \pi$, where r = (average diameter measured)/2, and $\pi = 3.14159$]. If a fluid-filled cavity was present in the luteal structure, volume of the cavity was calculated using the same procedure, and cavity volume was subtracted from the calculated total luteal volume.

2.9. Experiment 2: Statistical analyses

Progesterone concentrations in blood collected from cows on study Day −20, −17, −10, −3, and 0 were analyzed using the GLM procedure as in Experiment 1. Cycling status was determined as defined, and all pregnancy responses were examined using the GLIMMIX procedure model described in Experiment 1. Progesterone concentration categories were determined and analyzed as described in Experiment 1.

Diameter of the largest follicle present on study Days −17, −10, and −3 in the ovaries of a subset of cows (FL) was analyzed using the GLM procedure with treatment, parity, BCS (≤ 5 vs. > 5), median days postpartum (< 77 vs. ≥ 77), and two-way interactions of treatment and parity as fixed effects. The calculated volume of luteal tissue measured on study Days −17, −10, and −3 was examined using the same model. Treatment differences

were detected by F-test. Variance of treatment follicle diameters on study Days –17, –10, and –3 were compared as in Experiment 1.

2.10. Experiments 1 and 2: Combined statistical analyses

Cows were classified as anestrous before study Day –10 based on serum progesterone samples collected on Days –23 and –13 in Experiment 1 and Days –23, –17, and –13 in Experiment 2. Serum progesterone concentrations on study Day –10 for cows in both experiments were analyzed to determine the effect of either high or low serum progesterone concentration on subsequent pregnancy outcomes. A logistic model (GLIMMIX; SAS Inst. Inc.) was employed to determine the fixed effects of treatment, year, cycling status, BCS (≤ 5 vs. > 5), median days postpartum (<76 vs. ≥76), parity (primiparous vs. multiparous), progesterone concentration at study Day –10 (≥ 1 ng/mL [high] or < 1 ng/mL [low]), and the two-way interactions between cycling status and high vs. low progesterone concentration on study Day –10 on pregnancy per TAI. A similar model was used to examine differences in pregnancy per TAI when the four progesterone categories (defined previously for Experiment 1) were substituted in the previous model for high vs. low progesterone concentration on study Day –10.

3. Results

3.1. Experiment 1: Cycling status and estrus

The mean percentage of 1,496 cows that were cycling at the beginning of the experiment was 45.5% across herds and ranged from 16.4 to 69.5% by location (Table 1). Incidence of estrus as determined by estrus-detection patches revealed that 515 multiparous cows treated with PrePG on study Day –13 had a greater ($P < 0.05$) incidence of estrus between study Day –13 and –10 than 518 multiparous controls (32.3 vs. 15.4 %, respectively). In contrast, similar proportions of 137 treated and 136 control primiparous cows were detected in estrus (16.8 and 16.2%, respectively).

Incidence of estrus, as determined by estrus-detection patches between study Days -3 and 0, was greater ($P < 0.05$) for 452 treated multiparous cows than 454 multiparous controls (74.1 vs. 64.3%), whereas no difference was detected between 140 treated and 137 control primiparous cows (58.6, and 59.1%), respectively.

3.2. Experiment 1: Follicle diameters

Follicles ≥ 6 mm in diameter on study Days –10, –3, and 0 in cows at two locations were analyzed. The PrePG cows (n = 84) had larger ($P = 0.02$) follicles on study Day –10 than 87 controls (12.6 ± 0.5 vs. 11.0 ± 0.4 mm, respectively). No treatment difference in follicle size at study Day –3 ($P = 0.55$) or study Day 0 ($P = 0.37$) was detected. Differences in follicle diameter were detected ($P \leq 0.05$) between locations; however, no interaction between location and treatment was detected. Synchrony of follicle size did not differ between treatments because Levene's Test of Residuals detected no treatment difference in the variance of the largest follicle diameters on study Days –10, –3, and 0.

3.3. Experiment 1: LH responses to GnRH

The LH response to GnRH-1 and GnRH-2 was examined in 62 cows. At both sampling times, LH concentrations were greater ($P < 0.001$) 2 h after GnRH (Table 2). No differences in LH concentration were detected between treatments on either day, however, on study Day –10, anestrous cows had greater ($P = 0.005$) LH concentration 2 h after GnRH treatment than cycling cows (4.7 vs. 2.7 ng/mL, respectively).

3.4. Experiment 1:Progesterone and luteolysis

Concentrations of progesterone did not differ between treatments at study Day –13 ($P = 0.51$), –3, ($P = 0.35$), or 0 ($P = 0.37$; Fig. 2). In contrast, cows in PrePG treatment had lesser ($P < 0.001$) progesterone concentration on study Day –10 (3 d post PrePG treatment) than the control (0.5 ± 0.1 vs. 1.4 ± 0.1 ng/mL), respectively. In cows (n = 294) in which luteolysis was possible (progesterone ≥ 1 ng/mL) before the PrePG injection, the PrePG treatment (n = 142) increased ($P < 0.001$) the proportion of cows with luteolysis compared with 152 control cows (82 vs. 40%), respectively. Cows (n = 184) examined by transrectal ultrasonography were more ($P < 0.01$) likely to have new luteal tissue after PrePG (60%; n = 91) than the control (35%; n = 93). The PG injection administered to all cows on study Day –3 produced luteolysis in 98% of 910 cows that were assumed to have had a CL on study Day –3.

3.5. Experiment 1: Progesterone and pregnancy outcomes

Treatment of cows with PrePG altered the proportion of cows on study Day –10 having progesterone < 0.5 ng/mL and ≥ 4 ng/mL compared with those of the control (Fig. 3A). Resulting pregnancy per TAI was greater (P < 0.05) for PrePG than control cows when concentrations of progesterone were in the low-medium and high range (Fig. 3B). Overall pregnancy per TAI, however, did not differ between treatments (Table 3). Neither treatment nor BCS influenced pregnancy outcomes (Table 3). Multiparous cows were more (P < 0.05) likely to be pregnant 35 d after TAI (Table 3), and at the end of the season (96.1 vs. 92.3 %) than primiparous cows. Cows that were cycling at the beginning of the experiment were more (P = 0.004) likely to be pregnant at 35 d after TAI than anestrous cows (Table 3) as were (P = 0.033) cows that were > 70 d postpartum at TAI than later-calving cows (Table 3). Final pregnancy rate (95.1 vs. 93.7%) and pregnancy loss (1.4 vs. 1.4%) for PrePG and control cows, respectively, did not differ between treatments.

3.6. Experiment 2: Cyclicity and ovarian structures

Cyclicity status of cows by location ranged from 32.3 to 62.0% and averaged 49.9% (Table 1). Ovarian structures on study Day –17, –10, and –3 were analyzed in 169 cows at one location (FL). No differences in follicular diameter were detected between treatments at any time point examined. Neither BCS nor parity affected follicle size; however, 72 cows that were at ≥ 70 d postpartum at TAI had more (P = 0.007) total follicles ≥ 10 mm in diameter on study Day –17 than 82 cows that were < 70 d postpartum (1.1 ± 0.10 vs. 0.8 ± 0.1 per cow), respectively. Although total volume of

luteal tissue was similar between treatments on study Day –17, –10, and –3 in cows with at least one CL, more (P = 0.006) control cows (n = 86) had at least one CL on study Day –17 than PrePGG cows (n = 83; 18.6 vs. 4.8 %), respectively, 3 d after the $PrePGF_{2\alpha}$ was administered. On study Day –10 (7 d after PreGnRH was administered), more (P = 0.002) PrePGG cows (n = 83) had at least one CL than controls (n= 86; 63.9 vs. 41.9%), respectively. In addition, more (P = 0.022) PrePGG cows (n = 83) had at least one CL than controls (n = 86; 62.7 vs. 45.3 %), respectively, on study Day –3.

3.7. Experiment 2: Progesterone and luteolysis

Concentrations of progesterone did not differ between treatments on study Day –20 (P = 0.51), –10 (P = 0.39), –3 (P = 0.45), or 0 (P = 0.36; Fig. 4). In contrast, cows in PrePGG treatment had lesser (P = 0.02) progesterone concentration on study Day –17 (3 d after the PrePG treatment) than the control (0.37 ± 0.10 vs. 0.71 ± 0.10 ng/mL), respectively. In 180 cows in which luteolysis could occur (progesterone ≥ 1 ng/mL) after the PrePG injection, more (P < 0.001) PrePGG cows (n = 94) had luteolysis than did control cows (n = 86; 81 vs. 30%), respectively. The PG injection administered to all cows on study Day –3 produced luteolysis in 96% of 710 cows (349 controls and 361 PrePGG cows) that were assumed to have had a CL on study Day –3.

3.8. Experiment 2: Progesterone and pregnancy outcomes

Treatment of cows with PrePGG altered the proportion of cows with progesterone concentrations < 4 ng/mL compared with those of the control (Fig. 5A). Resulting

pregnancy per TAI was greater (P < 0.05) for PrePGG than control cows when concentrations of progesterone were in the low and low-medium range (Fig. 5B). Overall pregnancy per TAI did not differ between treatments (Table 4). Neither cycling status nor parity influenced TAI pregnancy outcome (Table 4). Cows ≥ 77 d postpartum at TAI were more (P < 0.001) likely to be pregnant 35 d after TAI (Table 4, and at the end of the season (85.6 vs. 79.1%), respectively, than cows < 77 d postpartum. Likewise, cows that had BCS > 5 were more (P < 0.001) likely to become pregnant than thinner cows (Table 4). Final pregnancy rates (82.6 vs. 82.6%) and pregnancy loss (5.4 vs. 3.6%) did not differ between PrePGG and control cows, respectively.

3.9. Experiments 1 and 2: Combined results

Combined results of the two experiments examined the relationship between progesterone status at the onset of the 7-d CO-Synch + CIDR program (study Day –10). Progesterone status had no effect (P = 0.47) on pregnancy outcomes of cycling cows (Fig. 6). In contrast, anestrous cows with low progesterone status at the onset of the TAI program had less (P < 0.05) pregnancy per TAI than cycling cows with low progesterone status as well as anestrous cows with high progesterone status.

Each of the four progesterone categories defined in Experiments 1 and 2 was examined for pregnancy risk using the low category progesterone concentration (<0.50 ng/mL) on study Day –10 as the referent (Table 5). The odds of pregnancy for the low-medium (0.50 to 1.99 ng/mL) and high-medium (2.00 to 3.99 ng/mL) progesterone categories did not differ from the referent. In contrast, cows in the high progesterone (≥

4 ng/mL) category were 1.38 times more ($P < 0.05$) likely to become pregnant than cows in the low progesterone category.

Pregnancy risk was less ($P < 0.05$) likely in the cows studied in 2012 than in 2011 (Table 5). In contrast, multiparous cows and cows with BCS > 5 were more ($P < 0.05$) likely to conceive to the TAI.

4. Discussion

Ovulation synchronization programs that utilize exogenous GnRH 10 d before TAI followed in 7 d with PG and a second GnRH injection either 24 h before (Ovsynch) or at TAI (CO-Synch) effectively facilitate fixed TAI in suckled beef cows without detection of estrus [17,18]. Addition of a progestin to the protocol improved the pregnancy response in anestrous cows [19,20]. These studies also produced pregnancy outcomes in anestrous cows that exceeded 50% at TAI; however, variability in follicle size and maturity at AI [7,10] probably limited the pregnancy response to TAI programs.

Prebreeding progesterone exposure also altered the subsequent pregnancy outcome, particularly when anestrous cows were compared with cycling cows [19,20]. Stage of the estrous cycle and its inherent differences in progesterone concentration are likely part of the variation observed in subsequent pregnancy outcomes. Diestrous (d 5 through 18 of the estrous cycle; theoretically 67% of cycling cows) cows in Experiment 1 in which luteolysis occurred in response to the PrePG injection should have been approximately d 0 of the cycle at the beginning of the CO-Synch program (study Day –10. Metestrous (d 1 to 4; theoretically 19% of cycling) cows should have been on d 4 to 7 of the cycle and proestrous and estrous (d 19 to 21; theoretically 14.3% of cycling cows) on d 1 to 3 of the

cycle. Therefore, nearly 100% of the cycling PrePG cows should have been on d 0 to 7 of their cycles on study Day –10. On study Day –10, 91% of the PrePG cows had concentrations of progesterone < 2 ng/mL compared with 78.7% of the controls (Fig. 3A).

In contrast, following the same logic as for Experiment 1, nearly 100% of the cycling PrePGG cows in Experiment 2 should have been on d 7 to 18 of their cycles on study Day –10. Only 28.9% of PrePGG cows, however, had progesterone concentrations ≥ 2 ng/mL compared with 19.9% of controls. Nonetheless, the proportion of control cows in the various progesterone categories on study Day –10 in both experiments was similar (Fig. 3A and 5A). Because approximately one-half of the cows were anestrous in Experiment 1 (45.5%) and Experiment 2 (49.9%), a limited number of cycling cows were available to test the hypothesis of the influence of high vs. low progesterone status at the onset of the TAI program.

Nevertheless, the cycling cows responded to the PrePG and PrePGG injections in predictable fashion. In Experiment 1, the proportion of cows with luteolysis was two-fold greater and the number of cows with a new CL on study Day –10 was nearly two-fold greater than in the control. Furthermore, diameter of the largest follicle 3 d after PrePG was greater and concentrations of progesterone were less than detected in controls, which is consistent with the PrePG cows being in proestrus or estrus on study Day –10.

In Experiment 2, more controls had a CL on study Day –17 (3 d after the PrePGG cows were administered PG) and concentrations of progesterone were greater than in the PrePGG than control cows. Luteolysis was also nearly three-fold greater in PrePGG cows by study Day –17 compared with controls. Although follicle diameters did not differ

between treatments in Experiment 2 on study Days −17, −10, and −3, more PrePGG than control cows had at least one CL at CIDR insert removal, indicating that more PrePGG than control cows ovulated in response to GnRH-1.

Elevated progesterone concentrations at the start of the Ovsynch protocol are associated with greater pregnancy per TAI in dairy cows [14,21]. In contrast, Perry et al. [11] suggested that low progesterone concentrations in beef cattle at the beginning of the TAI program may be favorable for increased pregnancy success. In the current studies, the presynchronization regimen was designed to increase the proportion of the cows that started the 7-d CO-Synch + CIDR protocol in either a low (Experiment 1) or high progesterone status (Experiment 2). Cycling cows in Experiment 1 were more likely to become pregnant to TAI than anestrous cows. In contrast, cyclicity had no effect on pregnancy per TAI in Experiment 2 when the additional GnRH injection was added to the presynchronization PrePG treatment used in Experiment 1.

Pregnancy outcomes in the two experiments based on defined progesterone categories in Fig. 3 and 5 are less than convincing that an ideal progesterone concentration range predisposes improved pregnancy outcome from the TAI. One consistency between experiments was the improved pregnancy outcome for cows in the range of 0.5 to 1.99 ng/mL at the onset of the TAI program. Combined results indicated, however, that cows having progesterone ≥ 4 ng/mL at the onset of the TAI program had increased pregnancy per TAI of 7.6 percentage points compared with cows having progesterone < 0.5 ng/mL. The previous result is likely a reflection of a large proportion of cycling cows in the former group and a large proportion of anestrous cows in the latter. Taken together, these experiments provide little evidence that concentration of progesterone on day of CIDR insertion and GnRH injection of the CO-synch + CIDR

program is likely to be predictive of subsequent pregnancy outcome to the TAI. Nonetheless, when the progesterone status on study Day –10 for the combined results was examined, previously anestrous cows with progesterone ≥ 1 ng/mL had better pregnancy outcomes than anestrous cows with progesterone < 1 ng/mL. In light of the latter observation, the probability of pregnancy is more likely related to not only progesterone concentration, but stage of follicular dominance and age of the CL at the time the TAI program is initiated.

Presynchronization with PG before initiating a TAI protocol in dairy cows improved oocyte quality [22] and pregnancy rates [23,24]. In beef cows, presynchronization (PG administered 3 d before initiating a 6-d CO-Synch + CIDR TAI protocol) improved follicle turnover in response to GnRH-1 and subsequent pregnancy success compared with a 5 d CO-Synch + CIDR treatment [11]. In addition, heifers were more likely to display estrus and had better follicle turnover after GnRH-1 when presynchronized with PG [12].

Ovarian characteristics at the initiation of a TAI protocol seem critical to treatment success. Varying proportions of cycling beef heifers on Days 2, 5, 9, 13, and 18 of their estrous cycles ovulated in response to GnRH (0, 75, 67, 50, and 58%, respectively) [25]. A large proportion of cows on D 15 through 17 of their estrous cycles failed to ovulate in response to GnRH [10]. Injection of a single dose of PG induced CL regression and estrus in the majority of cows between D 6 and 16 of the estrous cycle [26].

The GnRH-induced LH release is inhibited by greater circulating concentrations of progesterone and increased by elevated circulating concentrations of estradiol in the absence of progesterone [27]. The LH concentrations monitored in Experiment 1 supported the reported relationship between progesterone concentration and LH release

because greater LH release was detected in anestrous than cycling cows after GnRH-1 (Table 2). In contrast, the GnRH-induced LH release did not differ between anestrous and cycling cows after GnRH-2 when progesterone concentration averaged < 0.3 ng/mL in all cows.

Body condition is related to pregnancy outcomes in TAI programs [20,28]. In that study, a one-unit decrease in BCS resulted in a 22.9% decrease in pregnancy success. Conversely, we found no difference in pregnancy per TAI of cows with BCS > 5 compared with their thinner herdmates in Experiment 1. In contrast, during the subsequent year (Experiment 2), which was characterized by severe drought and poor pasture conditions, better conditioned cows had greater pregnancy per TAI than their thinner herdmates. It is possible that poorer BCS can be overcome by a more positive energy balance in postpartum cows [28]. Results from both experiments supported greater pregnancy success in suckled beef cows with a longer postpartum interval to TAI, which is consistent with previous conclusions [28,29].

In summary, presynchronization treatments changed serum concentrations of progesterone before initiating a TAI protocol. It seems beneficial for previously anestrous cows to start the 7-d CO-Synch + CIDR protocol in a high progesterone status. This can occur only if they can be induced to ovulate or exposed to supplemental progesterone sometime before the TAI protocol begins. Cows in our experimental conditions had better pregnancy outcomes when they were ≥ 75 d postpartum at TAI. Overall, pregnancy per TAI was not improved with the presynchronization regimen tested; however, consistent in both experiments was the improved pregnancy per TAI for cows starting the TAI program with progesterone in the 0.5 through 1.99 ng/mL range. Treatments that induce cyclicity in anestrous cows seem to have the potential to improve pregnancy

per TAI, whereas elevated progesterone concentrations before TAI in cycling cows had no benefit in terms of pregnancy success, as long as cows were cycling at the beginning of the TAI protocol. If cycling status could be determined or estimated before initiating a TAI protocol, perhaps treatments could be adjusted to reduce costs and improve pregnancy per TAI.

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Table 1
Selected characteristics of suckled beef cows enrolled in Experiments 1 and 2[1].

Location[1]	Breed	No. of cows	2-yr-olds, %	Mean days postpartum at AI	Mean BCS[2]	Mean cyclicity, %
		Experiment 1				
FL-1	Angus, Charolais, Brangus	228	10.5	69	5.0	30.3[3]
FL-2	Angus, Brangus	146	8.2	54	5.3	20.5[3]
GA-1	Angus	126	21.4	75	5.0	65.1[4]
KS-H	Angus x Hereford	195	25.1	80	5.7	53.3[4]
KS-C	Angus x Hereford	205	27.8	71	6.0	50.2[4]
KS-P	Angus, Hereford, Simmental	167	27.0	69	5.2	69.5[4]
SD-A	Angus x Hereford	222	37.8	74	4.4	22.7[4]
SD-C	Angus x Hereford	104	31.1	75	4.9	36.5[4]
SD-CT	Angus x Hereford	144	0.7	67	4.3	16.4[4]
		Experiment 2				
FL	Angus, Brangus	169	16.6	69	5.6	56.2[5]
KS-H	Angus x Hereford	195	37.4	80	5.5	32.3[5]
KS-C	Angus x Hereford	261	16.9	71	5.5	50.6[5]
KS-P	Angus, Hereford, Simmental	184	24.5	69	4.9	62.0[5]

[1] In Experiment 1, cows located in four states at nine locations were enrolled, and in Experiment 2, cows in two states and four locations were enrolled.
[2] Body condition score.
[3] Cyclicity determined from estrus-detection patches.
[4] Cyclicity determined by serum progesterone concentrations and estrus-detection patches.
[5] Cyclicity determined by serum progesterone concentrations only.

Table 2
Blood serum LH concentrations (ng/mL; least square means ± SEM) in cows sampled 0 and 2 h after GnRH-1 (D –10) and GnRH-2 (D 0; experiment 1).

		Study Day –10		Study Day 0	
Treatment[1]	n	0 h	2 h	0 h	2 h
Control	31	1.1 ± 0.4	3.3 ± 0.4[a]	1.1 ± 0.5	3.9 ± 0.5[a]
PrePG	31	1.2 ± 0.4	4.1 ± 0.4[a]	1.3 ± 0.5	3.0 ± 0.5[a]
Anestrus		1.4 ± 0.4	4.7 ± 0.4[a,A]	1.1 ± 0.4	2.8 ± 0.4[a]
Control	20	1.3 ± 0.5	4.1 ± 0.5[a]	1.0 ± 0.6	3.6 ± 0.6[a]
PrePG	17	1.5 ± 0.5	5.2 ± 0.5[a]	1.3 ± 0.6	2.0 ± 0.6[a]
Cycling		1.0 ± 0.4	2.7 ± 0.4[a,B]	1.3 ± 0.5	4.1 ± 0.5[a]
Control	11	1.0 ± 0.6	2.6 ± 0.6[a]	1.3 ± 0.8	4.3 ± 0.8[a]
PrePG	14	0.9 ± 0.6	2.9 ± 0.6[a]	1.4 ± 0.7	4.0 ± 0.7[a]

[a] Differs ($P < 0.001$) from 0 h within treatment-day.
[A,B] Differs ($P = 0.005$) between anestrous and cycling cows.
[1] See experimental design of treatments (Fig. 1).

Table 3
Pregnancy per timed AI in suckled beef cows (Experiment 1).

Item	%	n	P value
Treatment[1]			0.257
PrePG	55.5	765	
Control	52.2	770	
Cycling status			0.004
No	49.5	612	
Yes	60.5	500	
Parity			<0.001
Primiparous	48.2	331	
Multiparous	59.4	1204	
Days postpartum at AI			0.033
< 75	51.0	682	
≥ 75	56.7	854	
Body condition score			0.685
≤ 5	54.4	918	
>5	53.3	617	

[1] See experimental design of treatments (Fig. 1).

Table 4
Pregnancy per timed AI in suckled beef cows (Experiment 2).

Item	%	n	P value
Treatment[1]			0.951
PrePGG	44.4	402	
Control	44.0	401	
Cycling status			0.459
No	42.6	404	
Yes	49.1	399	
Parity			0.412
Primiparous	42.4	190	
Multiparous	46.0	613	
Days postpartum at AI			<0.001
< 77	36.1	263	
≥ 77	52.6	543	
Body condition score			0.008
≤ 5	39.6	340	
>5	49.8	463	

[1]See experimental design of treatments (Fig. 1).

Table 5
Pregnancy per timed AI is influenced by progesterone concentration on the day of controlled internal drug release (CIDR) insertion, year, days postpartum, parity, and body condition (Experiments 1 and 2 combined).

Item	n	Unadjusted mean %	Adjusted mean %	Odds ratio	95% CI
Progesterone category[1]					
< 0.50 ng/mL	1179	51.7	48.4	Referent	…
0.50–1.99 ng/mL	334	53.0	50.8	1.12	0.86 – 1.45
2.00–3.99 ng/mL	200	55.0	51.8	1.18	0.86 – 1.62
≥ 4.00 ng/mL	249	59.0	56.0	1.38	1.02 – 1.87
Year					
2011	1162	56.5	56.5	Referent	…
2012	800	48.4	47.0	0.73	0.57 – 0.93
Days postpartum[2]					
≤ 76	955	48.3	46.7	Referent	…
> 76	1007	57.9	56.8	1.48	1.22 – 1.79
Parity					
Primiparous	484	47.5	47.2	Referent	…
Multiparous	1478	55.1	56.3	1.44	1.16 – 1.81
Body condition[3]					
≤ 5	1011	50.8	49.3	Referent	…
> 5	951	55.7	54.2	1.26	1.01 – 1.59

[1] Concentration of progesterone before CIDR insertion in the CO-Synch + CIDR program (see Fig. 1).
[2] Median days between calving and day of timed AI.
[3] Body condition score assessed 1 wk before CIDR insertion.

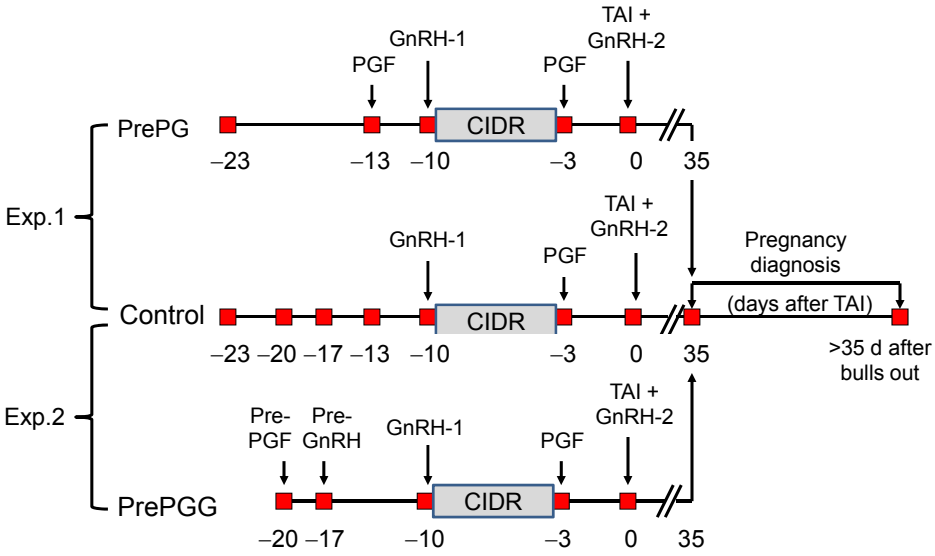

Fig. 1. Design of treatments administered in Experiments 1 and 2. The control cows (Experiment 1) were administered an injection of GnRH (GnRH-1) and insertion of a controlled internal drug release (CIDR) insert on study Day –10, an injection of $PGF_{2\alpha}$ (PGF) and CIDR removal on study Day –3, and an injection of GnRH (GnRH-2) and AI on study Day 0. The PrePG cows received an injection of PGF on study Day –13, then received the remaining experimental scheme as the controls. The PrePGG cows (Experiment 2) were administered an injection of PGF on study Day –20, an injection of GnRH on study Day –17, followed by the remaining experimental scheme as the controls. Blood samples were collected on study Day –23, –13, –10, –3, and 0 (Experiment 1) and study Day –20, –17, –10, –3, and 0 (Experiment 2).

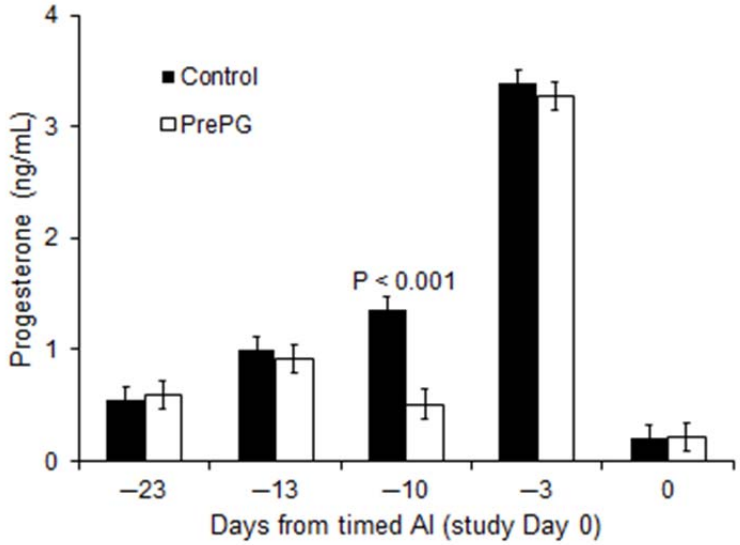

Fig. 2. Serum concentrations of progesterone in Experiment 1 on selected days relative to TAI (study Day 0) by treatment (see Fig. 1). Serum concentrations on study Day -3 also included progesterone supplied by controlled internal drug release (CIDR) insert, which was assumed to be 1 ng/mL after 7 d in situ. Numbers of samples in the control (range of 516 to 583) and in the PrePGG treatment (range of 514 to 579) varied because not all blood samples were collected at each location and some samples were missing.

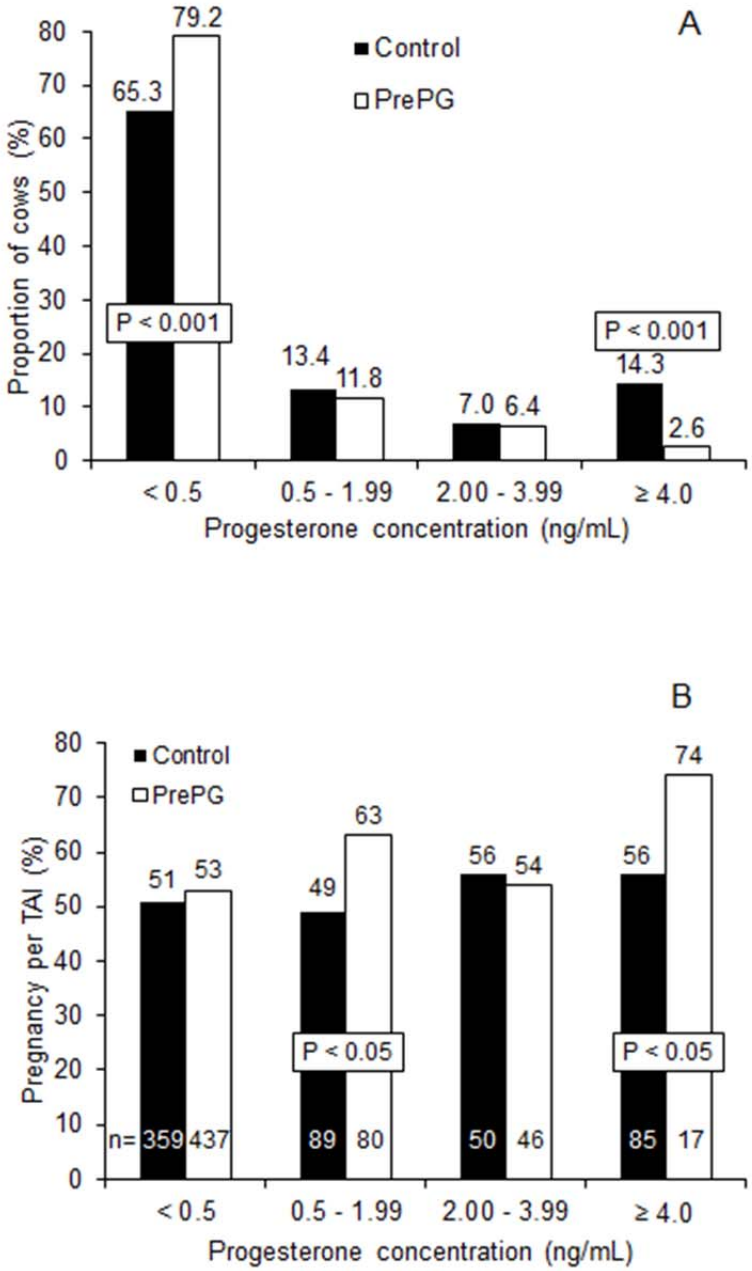

Fig. 3. Proportion of cows in selected progesterone categories (A) at the onset of the CO-Synch + CIDR timed AI protocol and resulting pregnancy per TAI (B). All cows received the CO-Synch + CIDR timed AI protocol (control), whereas PrePG cows received $PGF_{2\alpha}$ 3 d before the timed AI protocol (Experiment 1).

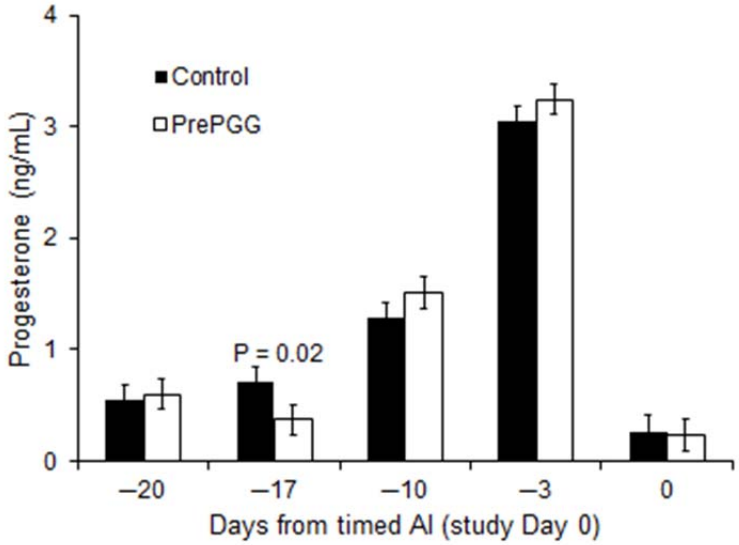

Fig. 4. Serum progesterone concentrations in experiment 2 on selected days relative to TAI (study Day 0) by treatment (see Fig. 1). Serum concentrations on study Day –3 also included progesterone supplied by controlled internal drug release (CIDR) insert, which was assumed to be 1 ng/mL after 7 d in situ. Numbers of samples in the control (range of 397 to 400) and in the PrePGG treatment (range of 400 to 402) varied because of missing blood samples.

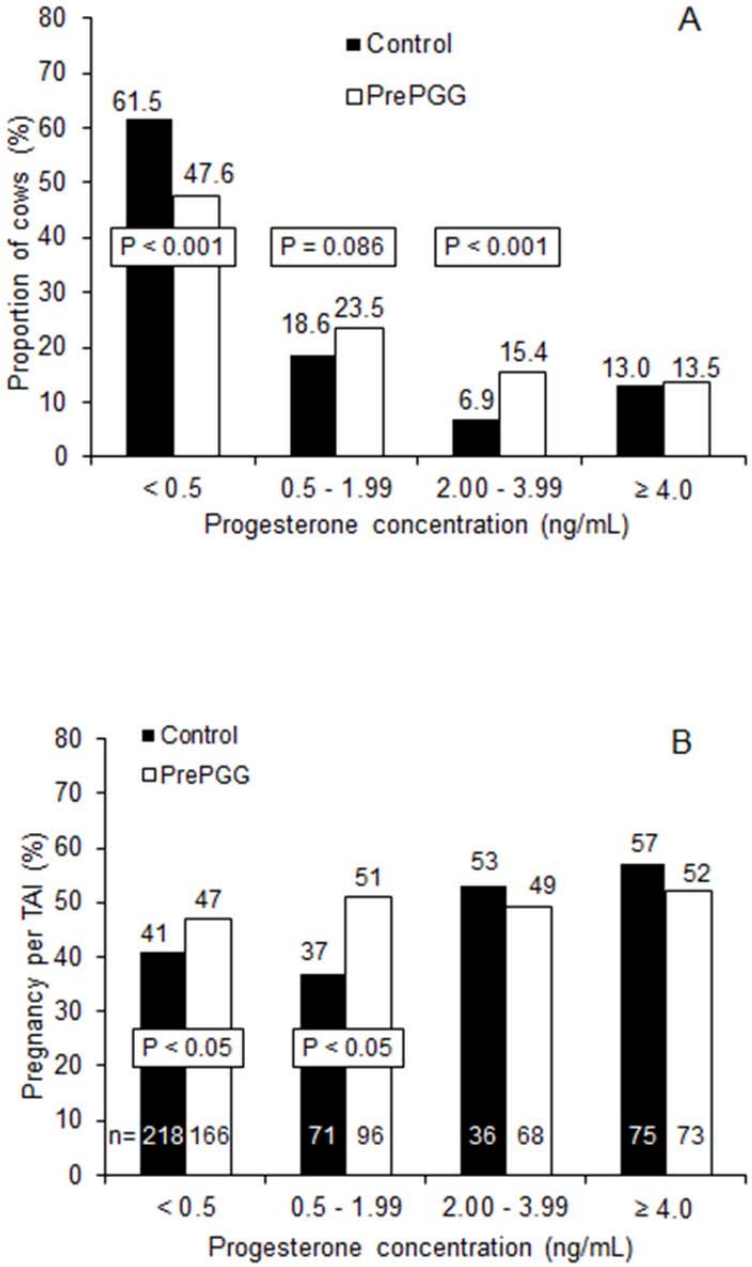

Fig. 5. Proportion of cows in selected progesterone categories (A) at the onset of the CO-Synch + CIDR timed AI protocol and resulting pregnancy per TAI (B). All cows received the CO-Synch + CIDR timed AI protocol (control), whereas PrePGG cows received $PGF_{2\alpha}$ 10 d and GnRH 3 d before the timed AI protocol (experiment 2).

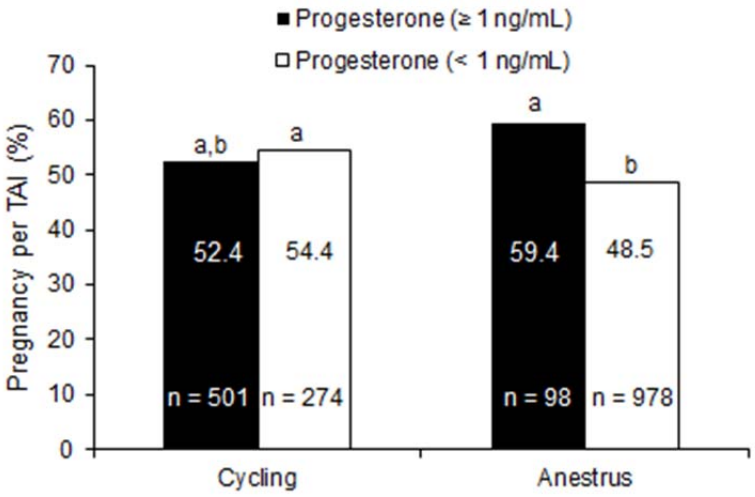

Fig. 6. Combined pregnancy per timed AI (TAI) for Experiments 1 and 2 based on serum progesterone concentrations and cycling status at the onset of the TAI protocol (study Day –10). Means without a common letter differ ($P \leq 0.05$).