Short communication: Change in dose delivery of prostaglandin $F_{2\alpha}$ in a 5-day timed artificial insemination program in lactating dairy cows¹

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ABSTRACT

We hypothesized that 50 mg of prostaglandin $F_{2\alpha}$ (PG) on d 6 would induce luteolysis in a traditional 5-d Ovsynch-72 program [GnRH 5 d before (d 0) and 72 h after (d 8) 25-mg PG doses (d 5 and 6 after GnRH); timed artificial insemination (AI) on d 8]. Experiment 1 monitored luteal regression of original and GnRH-induced luteal tissue (corpus luteum, CL) by transrectal ultrasonography and blood serum concentrations of progesterone after both 25-mg doses of PG (d 5 and 6; control; $n = 31$) or a single 50-mg dose of PG on d 6 ($n = 30$). Estrous cycles were presynchronized (GnRH 7 d before 25 mg of PG); 11 d later, cows were enrolled in a 5-d Ovsynch-72 program (62 to 71 d in milk) and treatments were administered. Blood was sampled for progesterone analysis and luteal structures were measured on d 0 (original CL) and d 5 through 9 to monitor original and new GnRH-induced CL. Control PG reduced luteal tissue area of original CL on d 6 and 7 compared with PG administered only on d 6, but no difference between treatments was detected by d 9. In contrast, no differences were detected in luteal tissue area of the induced CL on d 5 through 9. Serum progesterone on d 5 through 9 differed only on d 6 for control and the 50-mg dose. Luteolysis occurred in all 31 controls, but luteolytic failure occurred in 2 of 30 cows receiving 50 mg, in which no CL were present on d 0 but 1 or 3 new CL were present on d 5 in these 2 cows. Pregnancy outcome 32 d after AI was 14 of 30 (40%) compared with 15 of 30 (50%) for control versus 50-mg dose, respectively. Experiment 2 monitored luteolysis in nonpregnant repeat-service cows subsequently treated with the same 2 treatments as in experiment 1. Serum progesterone in 63 cows (serum progesterone ≥ 1 ng/mL on d 5) on d 5, 6, and 8 differed only on d 6 for control and the 50-mg dose. Luteolysis occurred in 32 of 34 controls and in 29 of 29 cows treated with 50 mg.

Pregnancy outcome 32 d after AI was 17 of 33 (52%) compared with 13 of 29 (45%) for control versus 50-mg dose, respectively. We concluded that the single 50-mg dose was equivalent to the control based on actual luteal tissue regression and decreased progesterone.

Key words: luteolysis, ovulation, luteal tissue, progesterone

Short Communication

Most timed AI (TAI) programs apply a combination of GnRH and prostaglandin $F_{2\alpha}$ (PG) to control follicular wave initiation, ovulation, and luteolysis in dairy herds before first and repeat AI. These programs generally consist of injecting GnRH (d 0), a standard 25-mg dose of PG on d 5 and 6 (5-d program) or a single dose of PG on d 7 (7-d program), GnRH at 56 h (Ovsynch-56) or 72 h (Co-Synch-72) after PG with TAI administered on d 8 (5-d program) or d 10 (7-d program). Unless PG is administered on d 5 and 6 in a 5-d program, luteolysis fails to occur in a proportion of cows that formed a new corpus luteum (CL) after GnRH administration on d 0 (Santos et al., 2010). Studies have demonstrated that a larger dose (200% of normal) of either PG (Ribeiro et al., 2012a) or a PG analog (Ribeiro et al., 2012a) given on d 5 failed to induce luteal regression equal to 2 standard doses of PG administered on d 5 and 6, thus compromising pregnancy per AI.

A recent report in nonlactating cows modified the 5-d program by applying a larger dose (250% of normal) of a PG analog on d 6 and compared that with standard doses of PG on d 5 and 6 (Valdecabres-Torres et al., 2013). Results indicated that the larger dose of PG on d 6 (a protocol that required less animal handling) produced luteolytic outcomes and final preovulatory follicle diameter similar to the standard doses of PG on d 5 and 6.

We hypothesized that administering 50 mg of PG to lactating dairy cows on d 6 would produce similar rates of luteolysis, as measured by decreased CL tissue area and serum progesterone, without compromising pregnancy outcomes. Our objective was to determine the effect of the standard control dose of PG on d 5 and 6 with a single larger (200% of control) dose of PG on d 6

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in lactating dairy cows before first postpartum AI (experiment 1) and before repeat services (experiment 2) on luteal tissue area and progesterone concentrations.

Experiment 1

In Experiment (Exp.) 1, estrous cycles were pre-synchronized [GnRH (2 mL of Factrel, Pfizer Animal Health, Madison, NJ) 7 d before 25 mg of PG (5 mL of Lutalyse, Pfizer Animal Health)] in 61 lactating Holstein cows (18 primiparous and 43 multiparous). Eleven days later, cows were enrolled randomly within parity in a 5-d Ovsynch-72 program (62 to 71 DIM) and treatments were administered as illustrated in Figure 1 (control cows: 25-mg dose of PG on d 5 and 6, n = 31; treated cows: single 50-mg dose of PG on d 6, n = 30). Cows were milked thrice daily and fed a TMR diet consisting of alfalfa hay, straw, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals balanced for 45 kg of 3.5% FCM. Standardized 150-d ECM yield for control and 50-mg cows were 49.5 ± 1.2 and 49.2 ± 1.2 kg/d, respectively. Weekly averages of daily milk yield and 305-d projected FCM and ECM also did not differ between treatments.

On d 0, follicles and original CL were mapped and measured by transrectal ultrasonography (5.0-MHz linear-array transducer, Aloka 500V, Corometrics Medical Systems Inc., Wallingford, CT). On d 5 through 9,

ovarian follicles, new GnRH-induced CL, and original CL were measured. The largest ovarian follicle on d 8 was traced back to its first appearance to determine the putative preovulatory follicle diameter. Spherical cavity-free area of luteal structures was calculated. Luteolysis was defined to occur when concentrations of progesterone were ≥ 1 ng/mL on d 5 and < 1 ng/mL on d 8. Blood serum was assayed by radioimmunoassay for progesterone (Stevenson et al., 2012) in both experiments. Assay sensitivity was 1.9 ± 0.5 pg/mL. Inter- and intraassay coefficients of variation for 4 assays were 6.5 and 7.9%, respectively.

Pregnancy was diagnosed by transrectal ultrasonography on d 32 after TAI. A positive pregnancy outcome required presence of anechoic uterine fluid and a CL ≥ 25 mm in diameter or anechoic uterine fluid and presence of an embryo with a heartbeat. Concentrations of progesterone and luteal tissue area were analyzed as repeat measures in procedure MIXED (SAS Inst. Inc., Cary, NC). Occurrence of luteolysis was analyzed by chi-squared test.

On d 0, 51 of 61 cows had at least 1 CL and 15 had 2 or more CL, whereas 10 cows (5 cows per treatment) had no CL. On d 5, 34 of 61 cows had at least 1 new CL and 5 cows had 2 or more new CL. Therefore, the ovulation response to GnRH on d 0 was 31 of 61 (50.8%). Numbers of cows with 1, 2, or ≥ 3 total CL (original plus new CL) on d 5 (1 CL: 12 vs. 13; 2 CL: 13 vs. 14; and ≥ 3 or more CL: 6 vs. 3) for control and 50-mg cows, respectively.

Original luteal tissue area was similar on d 5 but differed between treatments on d 6 ($P = 0.001$) and d 7 ($P = 0.009$), and tended ($P = 0.068$) to be less on d 8 for the control (Figure 2; upper panel). In contrast, no differences were detected between treatments for GnRH-induced luteal tissue area (Figure 2; lower panel).

Concentrations of progesterone differed ($P = 0.001$) only on d 6 between treatments (Figure 3; upper panel). Luteolysis occurred in all 31 controls, but failed to occur in 2 of 30 (6.7%) 50-mg cows in which no CL were present on d 0, but 1 or 3 new GnRH-induced CL were present on d 5 in the 2 cows with luteolytic failure.

Diameter of the dominant ovarian follicle on d 5 through 8 did not differ between treatments. Diameters of the dominant follicle on d 8 were 13.3 ± 0.5 vs. 13.5 ± 0.5 mm, respectively, for control and 50-mg cows.

Pregnancy outcomes were 12 of 30 (40%) for control cows and 15 of 30 (50%) for 50-mg cows (1 control was culled before pregnancy was determined).

Experiment 2

In Exp. 2, 80 cows diagnosed not pregnant to a previous AI were treated with GnRH on d 0 and assigned

Figure 1. Experimental scheme of treatments and measurements. In experiment 1, estrous cycles were presynchronized by injecting 100 μ g of GnRH and 25 mg of PG_{F_{2 α} (PG) beginning between 62 and 71 DIM. Cows were assigned randomly to receive either 25-mg doses of PG on d 5 and 6 or a 50-mg dose of PG on d 6. Ovarian structures were measured by transrectal ultrasonography (S) and mapped on d 0 and d 5 through 9. Both original corpora lutea (CL) on d 0 and GnRH-induced CL identified on d 5 were measured and monitored for diameter and luteal area (CL cavity area was deducted from total luteal area). Blood samples (B) were collected before each ovarian scan. TAI = timed AI. Color version available in the online PDF.}

Figure 2. Total luteal area of original corpus luteum (CL) tissue first identified on d 0 (upper panel) and total luteal area of GnRH-induced CL tissue first identified on d 5 (lower panel) in experiment 1. Control cows received 25-mg doses of PGF_{2α} (PG) on d 5 and 6, and treated cows received a 50-mg dose of PG only on d 6. Luteal tissue area differed between treatments as indicated by *P*-values.

randomly to the same 2 treatments as described in Exp. 1 (Figure 1). Blood was collected on d 0, 5, 6, and 8. Only data from 63 cows having serum progesterone ≥ 1 ng/mL on d 5 were analyzed in Exp. 2. Concentrations of progesterone and occurrence of luteolysis were analyzed as in Exp. 1.

Concentrations of progesterone differed between treatments only on d 6 (Figure 3; lower panel). Luteolysis occurred in all (29) 50-mg cows but failed to occur in 2 of 34 (5.9%) controls. Pregnancy outcomes at d 32 were 17 of 33 (52%) for control cows and 13 of

Figure 3. Concentrations of progesterone in control [25-mg doses of PGF_{2α} (PG) on d 5 and 6] and treated cows (50 mg of PG only on d 6) for cows in experiment (Exp.) 1 (upper panel) and Exp. 2 (lower panel). Concentrations differed (*P* = 0.001) between treatments only on d 6 in both experiments.

29 (45%) for 50-mg cows. One control cow was culled before pregnancy diagnosis.

In lactating dairy cows in the present study, reduction of CL tissue area (Exp. 1) and progesterone concentrations in both experiments resulting from the 50-mg dose of PG on d 6 was as effective as the standard split 25-mg doses on d 5 and 6. These results corroborate similar success reported in nonlactating cows (Valdecabres-Torres et al., 2013). Effective 5-d programs have been reported in which ovulation was synchronized in lactating dairy cows inseminated at first service when housed in freestall confinement (Bisinotto et al., 2010) or grazing operations (Ribeiro et al., 2011, 2012b). In those studies, timed AI occurred 72 h after PG, and GnRH was administered 12 to 18 h before timed AI or at the time of AI. In a 5-d program, a second dose of PG was given to ensure regression of any new luteal

structure that formed after GnRH-1-induced ovulation. Current industry application of the 5-d program applies a second PG dose (d 6) 24 h after the first PG dose (d 5).

Research results in 2,465 suckled beef cattle in 13 herds enrolled in a 5-d Co-Synch + controlled internal drug release (CIDR) program demonstrated that one 50-mg injection of PG at CIDR insert removal produced pregnancy outcomes similar to two 25-mg doses of PG administered at 0 and 8 h after CIDR insert removal (51 vs. 55%), respectively (Bridges et al., 2012).

Two recent experiments conducted in lactating dairy cows (Ribeiro et al., 2012a) in which estrous cycles were presynchronized before a 5-d Ovsynch protocol tested whether a single large dose (200% of control) of either cloprostenol (1 mg) or dinoprost (50 mg) administered on d 5 would produce acceptable rates of luteolysis, pregnancy, or both, compared with 2 standard split doses (0.5 or 25 mg, respectively) administered on d 5 and 6. In the first experiment, cows were treated with 0.5 mg of cloprostenol on d 5 and 6 (d 0 = GnRH-1 as in Figure 1) compared with 1 mg of cloprostenol (double dose) on d 5. More luteolytic failures occurred and pregnancy outcome was reduced by as much as one-third in cows receiving the 1-mg dose of cloprostenol in the first experiment. In the second experiment of similar design, a 50-mg dose of dinoprost administered on d 5 reduced pregnancy outcomes compared with the standard 25-mg doses administered on d 5 and 6.

The present study demonstrates that luteolysis is effective with the 50-mg dose administered on d 6 of a 5-d program as assessed by decreased luteal tissue area and decreased concentrations of progesterone. Application of the 50-mg dose also reduces cow handling. Application of the 50-mg dose on d 6 seems to be efficacious,

but testing pregnancy outcomes in a larger number of cows is warranted.

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