Short Communication: Pregnancy Rates to Timed Artificial Insemination in Holstein Heifers Given Prostaglandin F_{2α} Twenty-Four Hours Before or Concurrent with Removal of an Intravaginal Progesterone-Releasing Insert

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ABSTRACT

The objective was to compare pregnancy rates in nulliparous Holstein heifers given PGF_{2α} 24 h before, or concurrent with, removal of an intravaginal progesterone-releasing (CIDR) insert in 3 timed artificial insemination (TAI) protocols. Heifers (from 2 herds) were assigned randomly, over 11 mo, to 1 of 3 modified Ovsynch protocols. On d 0 (without reference to the stage of the estrous cycle), all heifers were given 100 µg of GnRH i.m. and a CIDR insert (containing 1.9 g of progesterone). In the PG-7/P4-8 protocol (n = 99), PGF_{2α} was given on d 7, and CIDR inserts were removed on d 8. In the PG-7/P4-7 (n = 98) and PG-8/P4-8 (n = 102) protocols, PGF_{2α} administration and CIDR removal occurred concurrently, on d 7 or 8, respectively. In all 3 protocols, a second GnRH treatment (100 µg) was given 48 h after PGF_{2α}, with TAI 16 to 20 h later. Blood samples were collected (subset of 124 heifers) on d 0, 7, 10 or 11 (i.e., at TAI), and 17. Pregnancy rates (32 d after TAI) for protocols PG-7/P4-8, PG-7/P4-7, and PG-8/P4-8 were 61.8, 55.6, and 54.1%, respectively. Pregnancy rate was higher when synchronization was initiated during diestrus than when initiated at other stages (57.0 versus 34.8%). Although pregnancy rates were not affected by season, there was an interaction between protocol and season; pregnancy rates were significantly lower in summer in heifers subjected to PG-7/P4-7 and PG-8/P4-8, but season did not affect pregnancy rates in heifers subjected to PG-7/P4-8. In summary, giving PGF_{2α} 24 h before CIDR removal, followed by TAI (PG-7/P4-8 protocol), resulted in consistent pregnancy rates, regardless of season, relative to protocols involving PGF_{2α} treatment concurrent with CIDR removal.

Key words: dairy heifer, timed artificial insemination, intravaginal progesterone insert, pregnancy rate

A protocol that yields good fertility to timed AI (TAI; without estrous detection) would be of considerable benefit to dairy producers. Although the Ovsynch protocol (Pursley et al., 1997) enables TAI and yields pregnancy rates comparable to other controlled breeding protocols in lactating dairy cows, pregnancy rates in nulliparous heifers were substantially lower than with alternative protocols (Schmitt et al., 1996; Pursley et al., 1997; Stevenson et al., 2000). In Ovsynch-treated heifers, failure of the first GnRH treatment to consistently induce ovulation and synchronize emergence of a new follicular wave and premature luteolysis (resulting in precocious estrus, before TAI) were potential contributors to poor fertility (Martinez et al., 1999, 2002; Moreira et al., 2000). In lactating dairy cows, the inclusion of an intravaginal progesterone (P4) insert (CIDR) in an Ovsynch-TAI protocol prevented premature estrus (Kim et al., 2003) and often improved pregnancy rates (Kim et al., 2003; El-Zarkouny et al., 2004; Stevenson et al., 2006) although not consistently (El-Zarkouny et al., 2004; Stevenson et al., 2006). The recommended protocol for estrus synchronization using a CIDR is to leave it in place for 7 d, give a luteolytic dose of PGF_{2α} or one of its analogs on the sixth day, and remove the CIDR 24 h after (d 7) PGF_{2α} treatment (Canadian Animal Health Institute, 2005). In a previous study, Ambrose et al. (2005) incorporated a CIDR into the Ovsynch protocol for 8 d in dairy heifers, based on the premise that removing the insert 24 h after treatment with PGF_{2α} would reduce precocious estrus and tightly synchronize ovulation. Although a pregnancy rate of approximately 60% was attained, heifers had to be handled 4 times before TAI. In a related study, Hittinger et al. (2004) reported that giving PGF_{2α} concurrent with CIDR removal (on d 7 or 8) reduced animal handling without compromising synchrony. This conclusion was based on visual detection of estrus and confirmation of

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ovulation by ultrasonography; however, that study was not designed to determine pregnancy rates. Therefore, the objective of the present experiment was to compare pregnancy rates to TAI in Holstein heifers treated with a CIDR for 7 or 8 d, and given PGF_{2α} 24 h before, or concurrent with, CIDR removal, in 3 modified Ov-synch protocols.

Nulliparous Holstein heifers, 15.8 ± 1.5 mo (mean ± SEM) of age, from 2 herds, located approximately 20 km apart, were used. Heifers were group-housed on partly sheltered dirt lots and were fed barley silage plus rolled barley (16% protein; approximately 1.8 kg/head per day on an as-fed basis) once daily. Hay (second-cut alfalfa, timothy, and orchard grass mixture), water, iodized salt, and a mineral supplement were available ad libitum. Daily forage consumption was estimated to be 9.0 kg (DM) per head. No seasonal adjustments were made to the rations.

The study was conducted over a period of 11 mo, encompassing fall (September to November), winter (December to February), spring (March to May), and summer (June to August), with no TAI in July. For these seasons, mean temperatures were 4.6, -8.5, 3.6, and 17.0°C, respectively (range, -35.4 to +34.9°C). Cohorts were started at approximately monthly intervals, with synchronization treatments initiated concurrently at the 2 farms. All animal handling procedures were in accordance with the Canadian Council on Animal Care guidelines and were approved by the institutional animal care committee of the University of Alberta.

Heifers, at unknown stages of the estrous cycle, were randomly assigned to 1 of the 3 experimental protocols (Figure 1): PG-7/P4-8 (n = 99), PG-7/P4-7 (n = 98), or PG-8/P4-8 (n = 102). On d 0, all heifers received an intravaginal CIDR insert (1.9 g of P4; Pfizer Animal Health, Orangeville, Ontario, Canada), with GnRH (100 µg of gonadorelin acetate; Fertiline; Vetoquinol, Lavaltrie, Quebec, Canada) concurrently given i.m. Heifers in the PG-7/P4-8 group received PGF_{2α} (25 mg of dinoprost tromethamine; Lutalyse, Pfizer Animal Health) on d 7, and the CIDR insert was removed on d 8. In the PG-7/P4-7 group, the CIDR insert was removed on d 7, and PGF_{2α} was given concurrently. Similarly, in the PG-8/P4-8 group, the CIDR insert was removed on d 8, with concurrent PGF_{2α} treatment. In all protocols, the second GnRH treatment was given 48 h after PGF_{2α}, with TAI 16 to 20 h thereafter. Consequently, TAI was done on d 10 in PG-7/P4-7 and PG-7/P4-8 protocols but on d 11 in the PG-8/P4-8 protocol. Frozen-thawed semen from registered Holstein sires was used for TAI (sires were equally distributed among the 3 protocols). Two technicians, 1 per location, performed all inseminations. At the insistence of one of the cooperating producers, any heifer observed in standing

estrus in the 24-h period before the scheduled TAI was inseminated within 2 h of detected estrus. Any heifer that was detected in standing estrus >24 h after TAI was reinseminated and considered nonpregnant to TAI. Heifers that were not detected in estrus and rebred, but were not pregnant at pregnancy diagnosis, were assigned to a treatment different from the previous one (maximum of 2 additional inseminations).

Blood samples were collected from a subset of approximately 40% of the heifers from each location (n = 124; approximately equal numbers from each protocol), on d 0, 7, 10 or 11 (i.e., at TAI), and 17. Samples were collected from the coccygeal vessels into heparinized tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), centrifuged (20 min, 1,500 × g), with plasma harvested, and stored at -20°C until analyzed. Plasma P4 concentrations were determined by radioimmunoassay (Coat-A-Count P4 Invitro Diagnostic Test Kit, Diagnostic Products Corporation, Los Angeles, CA). All samples were tested in duplicate, and the intrassay coefficient of variation was <3% (lowest detection limit, 0.1 ng/mL). Plasma P4 concentrations were used to determine the luteal status of heifers at the first GnRH injection and to determine the response to synchronization treatments. If plasma P4 concentrations were ≥2.0 ng/mL on d 0, heifers were considered diestrous at the initiation of the synchronization protocol. Furthermore, heifers with P4 concentrations ≥1.0 ng/mL on d 7, <1.0 ng/mL on d 10 or 11 (at TAI), and ≥1.0 ng/mL on d 17 were considered to have responded to the synchronization treatment.

Pregnancy diagnosis was performed 31 or 32 d after TAI by transrectal ultrasonography, using a real-time ultrasound scanner equipped with a 7.5-MHz linear-array transrectal transducer (Aloka 500-V, Aloka Corporation, Tokyo, Japan).

Data were analyzed by GENMOD procedures of SAS (Version 9.1 for Windows, SAS Inst. Inc., Cary, NC). The statistical model first determined the main effect of treatment (TAI protocol) on pregnancy rate. Later, the main effects of location (farm), season, and AI type, and their interactions with treatment, were added to the model. The main effects of synchronization status and luteal status at initiation of GnRH were also considered in the subset of cows in which plasma P4 concentrations were determined. When differences or trends of main effects were evident, contrast estimates were applied in the GENMOD procedure of SAS. Differences were declared significant at $P < 0.05$, whereas $P > 0.05$ but ≤ 0.10 was considered a trend.

Thirty-five heifers were assigned to TAI protocols twice and 11 heifers thrice; no heifer was subjected to the same protocol more than once. Three heifers were detected in estrus and reinseminated within 48 h of

Figure 1. Schematic diagram of timed AI (TAI) protocols. All heifers were given GnRH i.m. and an intravaginal progesterone-releasing (CIDR) insert on d 0. In the PG-7/P4-8 protocol ($n = 99$), $\text{PGF}_{2\alpha}$ was given on d 7 and CIDR inserts were removed 24 h later (d 8). In the PG-7/P4-7 ($n = 98$) and PG-8/P4-8 ($n = 102$) protocols, $\text{PGF}_{2\alpha}$ administration and CIDR removal occurred concurrently, on d 7 or 8, respectively. In all 3 protocols, the second GnRH treatment was given 48 h after $\text{PGF}_{2\alpha}$, with TAI performed 16 to 20 h later. Blood samples were collected from a subset of 124 heifers on d 0, 7, 10 or 11 (at TAI), and 17.

TAI, 1 within 5 d and 17 within 3 wk; all were considered nonpregnant to TAI.

When only the treatment (TAI protocol) effect was considered, pregnancy rates did not differ ($P = 0.50$). However, when the effects of location, season, AI type, and the various interactions were included in the statistical model, treatments tended to differ ($P = 0.06$). When contrast estimates were applied, heifers in the PG-7/P4-8 group had higher pregnancy rates than those in the PG-8/P4-8 group ($P = 0.03$), but pregnancy rates did not differ ($P = 0.11$) between heifers in the PG-7/P4-8 and PG-7/P4-7 (Table 1). There was no difference in pregnancy rates between heifers treated with the PG-7/P4-7 or PG-8/P4-8 protocols. In a previous study (Hittinger et al., 2004), giving $\text{PGF}_{2\alpha}$ at CIDR removal (either 7 or 8 d after the initial GnRH and CIDR inser-

tion) did not affect luteal regression and synchrony of estrus compared with giving $\text{PGF}_{2\alpha}$ 24 h before CIDR removal. Furthermore, intervals from CIDR removal to ovulation, and from standing estrus to ovulation, were not significantly different when the CIDR was removed either concurrent with $\text{PGF}_{2\alpha}$ or 24 h later (Hittinger et al., 2004). However, pregnancy rates were not determined in that study. The pregnancy rates achieved with the PG-7/P4-8 protocol in the present study were consistent with a 58.7% pregnancy rate to an identical protocol used in dairy heifers that we previously reported (Ambrose et al., 2005).

Protocol PG-7/P4-8 resulted in higher pregnancy rates compared with protocol PG-8/P4-8, and a nonsignificant improvement ($P = 0.11$) over protocol PG-7/P4-7. We attributed these differences to ovarian follicular

Table 1. Pregnancy rates (PR) in all heifers (n = 299), in heifers that were subjected to timed AI (TAI; n = 242), in a subset of heifers with progesterone (P4) data (n = 124), response rates to synchronization protocols, and PR in heifers considered to have responded to the synchronization treatment (responders; n = 115)

Protocol	All heifers ¹ PR (n)	TAI heifers PR (n)	P4 subset PR (n)	Response rate n (%)	Responders PR (n)
PG-7/P4-8	61.8 ^{a,x} (99)	60.0 (85)	62.8 (43)	39/43 (90.7)	68.4 (39)
PG-7/P4-7	55.6 ^y (98)	52.5 (80)	52.5 (40)	38/40 (95.0)	55.3 (38)
PG-8/P4-8	54.1 ^b (102)	54.6 (77)	43.9 (41)	38/41 (92.7)	47.4 (38)

^{a,b}P = 0.03.^{x,y}P = 0.11.¹Includes heifers that were TAI and that were inseminated after detected estrus. Pregnancy rate did not differ when only treatment effects were considered; however, the difference became evident when the statistical model included location, season, and AI type (TAI or AI after detected estrus).

dynamics. Assuming that ovulation occurred approximately 30 h after GnRH treatment, the interval between CIDR removal and the expected time of ovulation was ~54 h in the PG-7/P4-8 protocol, whereas it was 24 h longer (~78 h) in the PG-7/P4-7 and PG-8/P4-8 protocols. Hence, in the PG-7/P4-8 protocol, preovulatory follicles had the shortest interval of growth in a low-P4 environment (i.e., from CIDR removal to ovulation). Furthermore, assuming that a new follicular wave emerged approximately 1.5 d after the initial GnRH treatment (Martinez et al., 1999) in most heifers, the postinsemination ovulation would have occurred approximately 8.8 d after follicular emergence in the PG-7/P4-8 and PG-7/P4-7 groups, versus 9.8 d in heifers in the PG-8/P4-8 group. Although the interval between expected follicular emergence and ovulation did not differ between the PG-7/P4-8 and PG-7/P4-7 protocols, overall pregnancy rates tended to be lower in heifers assigned to the latter protocol (they were significantly lower during the summer; Figure 2). Considering that the interval from CIDR insertion to removal (length of P4 exposure) was similar for the PG-7/P4-8 and PG-8/P4-8 protocols, we inferred that the short interval from CIDR removal to ovulation was beneficial to preovulatory follicles in the PG-7/P4-8 group, whereas longer intervals from CIDR removal to ovulation may have compromised oocyte quality in the PG-7/P4-7 and PG-8/P4-8 groups (especially during summer). On the contrary, pregnancy rate was reduced in dairy heifers when the second GnRH treatment was given 24 h after PGF_{2α} in a TAI protocol with no P4 insert (Schmitt et al., 1996). However, when the interval between PGF_{2α} and the second GnRH treatment was increased to 48 h, pregnancy rates were comparable to that after insemination at detected estrus. Thus, although shortening the 48-h interval between PGF_{2α} and the second GnRH treatment may compromise fertility, creating a low-P4 environment (as with a CIDR insert left in place for 24 h after the luteolytic PGF_{2α} treatment) appeared to improve fertility. We inferred

that this improvement may have been due to more synchronous ovulation or improved oocyte competence. Additional studies are required to verify the fertility improvement in the PG-7/P4-8 protocol and determine its cause.

There is a paucity of reports on the inclusion of a CIDR in an Ovsynch-type TAI protocol for dairy heifers. Rivera et al. (2005) inserted a CIDR device containing 1.38 g of P4 on d 0 (at first GnRH) and removed it 6 d later, after giving PGF_{2α}, and heifers were inseminated 48 h later, concurrent with the second GnRH treatment (Co-synch). The overall pregnancy rate in that study was only 32%, which was attributed to a poor insemination technique adopted by 2 of the 3 inseminators. In another study, Peeler et al. (2004) reported pregnancy rates of approximately 58% in heifers that were subjected to Ovsynch-TAI protocols that included a CIDR containing 1.38 g of P4. In that study, estradiol cypionate (ECP) was used in place of the first GnRH treatment to initiate follicular wave emergence, and the CIDR insert was removed after 7 d, with PGF_{2α} given concurrently. One group of heifers received ECP 24 h after CIDR removal, whereas another group received GnRH 48 h after CIDR removal. The latter group of heifers was further divided into 3 subsets for TAI at 48 (Co-synch; concurrent with GnRH treatment), 56, or 72 h after CIDR removal; pregnancy rates did not differ (56.5, 60.0, and 56.5%, for the 3 groups, respectively). Heifers that received ECP 24 h after CIDR removal were also subjected to TAI at 48, 56, or 72 h after CIDR removal. Pregnancy rates were 66.7, 81.8, and 56.6%, with heifers in the 56-h group having a higher pregnancy rate than the other 2 groups. Although differences were significant, the relatively small numbers of heifers assigned to the 6 treatment groups (range, 10 to 23 per group; only 11 heifers constituted the group that had the highest pregnancy rate of 81.8%) was a limitation in the work published by Peeler et al. (2004).

Based on P4 concentrations, the overall synchronization rate (92.7%; Table 1) seemed higher than in previ-

Figure 2. Effect of season on pregnancy rates for the PG-7/P4-8, PG-7/P4-7, and PG-8/P4-8 protocols. Heifers were assigned nearly equally within timed AI (TAI) protocols, with 58, 99, 69, and 73 heifers inseminated during fall, spring, summer, and winter, respectively. There was an interaction between season and TAI protocol ($P = 0.02$), with a higher pregnancy rate for the PG-7/P4-8 protocol during the summer, compared with the PG-7/P4-7 and PG-8/P4-8 protocols. Refer to Figure 1 for definition of protocols PG-7/P4-8, PG-7/P4-7, and PG-8/P4-8.

ous studies (84 and 86%; Lucy et al., 2001 and Rivera et al., 2005, respectively) that used a CIDR for synchronization of estrus or ovulation in dairy heifers. When only these synchronized heifers ($n = 115$) were considered, differences among groups in pregnancy rates were slightly exaggerated (Table 1) but were not significantly different, due to limited statistical power.

Pregnancy rates were higher ($P = 0.02$) in the present study when synchronization protocols were started during diestrus (57.0%) versus all other stages of the estrous cycle (34.8%). Similarly, in a previous study (Moreira et al., 2000), pregnancy rates were highest (75%) when the initial GnRH treatment was given on d 10 of the estrous cycle, coinciding with the approximate time of emergence of the second follicular wave in cattle (Ginther et al., 1989), or perhaps due to exposure to higher concentrations of P4 before PGF_{2α} treatment (Folman et al., 1984). However, that study (Moreira et al., 2000) involved a small number ($n = 24$) of heifers, and the pregnancy rate (75%) was based on only 4 heifers.

There was no main effect of season on pregnancy rates; however, there was an interaction between season and TAI protocol ($P = 0.02$; Figure 2). Heifers subjected to the PG-7/P4-7 and PG-8/P4-8 protocols had lower pregnancy rates in summer compared with other seasons, whereas there was no significant effect of season on pregnancy rate in heifers assigned to the PG-7/P4-8 protocol. The mean summer temperature was 17°C, and the mean peak temperature during the summer was 33°C. Although the detrimental effects of high ambient temperature on dairy cattle fertility in north-

ern climates may be debatable, we recently reported (Ambrose et al., 2006) that heat stress contributed to poor fertility in lactating dairy cows in this geographic area (53°33' N and 113°28' W). The negative effects of heat stress on fertility in dairy cows (Folman et al., 1983; Hansen, 1997) are well known; however, high environmental temperatures also had detrimental effects on conception rates in nulliparous dairy heifers (Donovan et al., 2003). High ambient temperatures decreased fertility and plasma P4 concentrations during the luteal phase in dairy cows (Folman et al., 1983). Hence, protocols that ensured high circulating P4 may be beneficial during summer season. In this regard, protocols PG-7/P4-8 and PG-8/P4-8 both had longer (8 d) P4 exposure; however, pregnancy rates were higher during summer only in the PG-7/P4-8 protocol. We inferred that the short period of low-P4 exposure (i.e., interval between CIDR removal and ovulation) was particularly beneficial when the ambient temperature was high. Whether similar results would have emerged with the use of a CIDR with lower P4 content, such as the ones currently marketed in the United States, is not known.

Although TAI was scheduled for all heifers, at 1 location, the producer actively detected estrus during the 24-h period before scheduled TAI. Fifty-three of the 222 heifers were detected in estrus (16, 17, and 20 heifers for the PG-7/P4-8, PG-7/P4-7, and PG-8/P4-8 protocols, respectively) approximately 12 h before the scheduled TAI and inseminated within 2 h after detected estrus. Three heifers (1 from each protocol) were detected in estrus and inseminated approximately 6 h before the scheduled TAI. Whether detected in estrus or not, all heifers received the second GnRH treatment at their assigned time. At the second location, there was no preplanned estrous detection, but a single heifer (1 of 77) that was noted in estrus approximately 12 h before TAI was inseminated within 1 h after detected estrus. The pregnancy rates in heifers inseminated at standing estrus were not different ($P = 0.11$) from that in heifers inseminated at a fixed time (63.2 versus 55.8%, respectively).

In 2 of the 299 (0.7%) heifers, the CIDR was missing at the scheduled time of its removal and was later found on the ground by the producer. This rate of loss (0.7%) seemed lower than the 5% loss reported for dairy heifers (Lucy et al., 2001) but was similar to that reported (1% loss) for beef cows in the same study. One of the heifers that lost the CIDR was detected in estrus and was inseminated 12 h before scheduled TAI, whereas the other heifer was subjected to TAI. Both heifers were confirmed pregnant. Neither of these heifers was excluded from the analysis.

In conclusion, dairy heifers given PGF_{2α} 24 h before CIDR removal (PG-7/P4–8 protocol) had higher pregnancy rates than those assigned to the PG-8/P4–8 protocol. In summer, the PG-7/P4–8 protocol yielded significantly higher pregnancy rates than with the PG-7/P4–7 and PG-8/P4–8 protocols in which PGF_{2α} was given concurrent with CIDR removal.

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