Comparison of Two Estrus-Synchronization Protocols and Timed Artificial Insemination in Dairy Cattle

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ABSTRACT

The objective of this study was to evaluate the effect of the Ovsynch protocol with and without exogenous progesterone on pregnancy rate (PR) in cows in which estrous cycles were previously synchronized with 2 doses of PGF_{2α} and that were not detected in estrus during the presynchronization period. The study was conducted in Chihuahua, Mexico (8,650 Holstein milking cows; 305-d mature equivalent milk yield = 13,790 kg). On d 47 postpartum, estrous cycles in cows were synchronized by using 2 doses of PGF_{2α} 14 d apart. Any cow detected in estrus during this presynchronization period was inseminated. Cows not detected in estrus were selected at random and assigned to receive progesterone supplementation or to serve as controls. Controls (n = 594) were subjected to the Ovsynch protocol and cows in the progesterone supplemented treatment (n = 594) were subjected to the Ovsynch protocol plus an intravaginal insert containing 1.9 g of progesterone inserted at the time of the first GnRH injection and removed 7 d later. Progesterone-supplemented cows had a greater PR (31.2%) compared with controls (22.7%). Plasma progesterone concentrations at artificial insemination (AI) were <1 ng/mL and did not differ between treatments. At 14 d post-AI, however, more cows that received progesterone supplementation had concentrations of progesterone >1 ng/mL compared with controls. It was concluded that after a presynchronization period, cows subjected to the Ovsynch program and supplemented with exogenous progesterone had a greater PR and greater concentrations of progesterone after AI than those subjected to the Ovsynch protocol and not supplemented with progesterone.

Key words: Ovsynch, progesterone, fertility, pregnancy rate

INTRODUCTION

Timed AI (TAI) and estrus-synchronization protocols have become a popular reproductive strategy to im-

prove pregnancy rate (PR) in modern dairy cattle operations (Pursley et al., 1995; Burke et al., 1996; Stevenson et al., 1999). The advantage of these protocols is that detection of estrus is not necessary. Therefore, TAI has become crucial in herds in which detection of estrus is difficult, especially in large dairy herds.

The Ovsynch protocol can be started at any time of the estrous cycle of the cow. It involves administration of 1 dose of GnRH, followed in 7 d by PGF_{2α}, and a second dose of GnRH 2 d after PGF_{2α}, and subsequent TAI 12 to 24 h later. The protocol synchronizes ovarian follicular waves and time of ovulation (Pursley et al., 1995). Studies using the Ovsynch protocol have shown PR between 30 and 40% (Pursley et al., 1995; Burke et al., 1996; Stevenson et al., 1999). It has been demonstrated that ovulation was synchronized more precisely and fertility was improved when the Ovsynch protocol was started between d 5 and 12 of the estrous cycle (Vasconcelos et al., 1999; Cartmill et al., 2001). Subsequently, it was shown that the administration of 2 doses of PGF_{2α}, 14 d apart (Presynch) placed a large proportion of cows between d 5 and 12 of the estrous cycle when the Ovsynch program was started 12 d after the second dose of PGF_{2α} of this protocol (Moreira et al., 2001).

Other derivations of these original protocols have been developed during the last few years. When an intravaginal insert containing progesterone (P₄) was administered for 7 d between the first dose of GnRH and the administration of PGF_{2α} of the Ovsynch protocol, conception rate to TAI was improved (El-Zarkouny et al., 2004; Ambrose et al., 2005; Stevenson et al., 2006). A reproductive protocol using Presynch combined with detected estrus and subsequent Ovsynch with P₄ supplementation for cows not identified in estrus has not been reported previously.

The hypothesis of this study was that supplementing cows with P₄ via an intravaginal insert during the Ovsynch protocol would improve PR in cows not previously observed in estrus during a presynchronization period. The objective of this study was to evaluate, in a large dairy herd in Mexico, the effect of the Ovsynch protocol with exogenous P₄ on PR in cows that were previously

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subjected to the Presynch protocol, but were not observed in estrus during the presynchronization period.

MATERIALS AND METHODS

Cows and Herd Management

The study was conducted in a large dairy herd in Chihuahua, Mexico, between January and December 2005. This herd consisted of 8,650 milking cows with a mature equivalent 305-d milk yield of 13,790 kg. Lactating cows were housed in dry lots, milked, and fed a TMR 4 times daily to meet or exceed the nutritional requirements of NRC (2001). Cows were dried off between 50 and 70 d before expected parturition and maintained in a dry lot (far-off cows) until 21 d before expected parturition. Prepartum transition (close-up) cows (21 d before expected parturition) were housed in a dry lot with adequate feed bunk space and shade.

Within 12 h postpartum, cows were routinely evaluated. This evaluation consisted of BCS (scale of 1 to 5) and mammary gland evaluation for clinical mastitis (strip cup). Determination of retained fetal membranes was conducted at 24 h postpartum. If fetal membranes were retained and fever occurred ($>39.5^{\circ}\text{C}$), cows were treated with a systemic antibiotic daily for 5 d (oxytetracycline i.v., 15 mg/kg of BW). After processing, cows were moved to a postpartum lot for approximately 21 d. At 60 DIM cows were given 500 mg of bST s.c. every 11 d until 200 DIM. The voluntary waiting period was 47 d. On d 47, cows were subjected to a presynchronization protocol using 2 doses of $\text{PGF}_{2\alpha}$ 14 d apart (Pre-synch; Moreira et al., 2001). Any cow detected in estrus after administration of either of the 2 doses of $\text{PGF}_{2\alpha}$ was inseminated at estrus according to the a.m.–p.m. rule. Cows not detected in estrus during that period were subjected 12 d later to the Ovsynch protocol (Purley et al., 1995). Pregnancy rate at this synchronized ovulation was reported to be 19.5% in a previous study in the same dairy herd (Melendez et al., 2006).

Experimental Protocol

Controls were those cows subjected to the Ovsynch protocol that had not been previously identified in estrus during the presynchronization period (**Pre + Ov**). Treatment cows were subjected to the Ovsynch protocol and also received a P_4 (1.9 g) intravaginal insert given at the time of the first GnRH and removed 7 d later (**Pre + Ov + P_4**). To detect an increase in PR from 20% to 27% (95% confidence; 80% power), a sample size of 452 cows/treatment was required (Win Episcopo, 2000).

At d 73 postpartum, Pre + Ov cows ($n = 594$; 203 primiparous and 391 multiparous cows) received a dose of GnRH (100 μg of gonadorelin, i.m.), a dose of $\text{PGF}_{2\alpha}$

(500 μg of cloprostenol, i.m.) 7 d later, a second dose of GnRH (100 μg of gonadorelin, i.m.) 2 d later, and TAI 16 to 24 h later.

Cows from the Pre + Ov + P_4 treatment ($n = 594$; 203 primiparous and 391 multiparous cows) were matched randomly by parity using frequency matching based on farm records to obtain a similar number of primiparous and multiparous cows per treatment. These cows also started the Ovsynch protocol at 73 d postpartum, and a P_4 -releasing intravaginal insert (1.9 g of P_4) was inserted at the time of the first GnRH dose and removed 7 d later. Cows also were given a dose of $\text{PGF}_{2\alpha}$ when the intravaginal insert was removed. Cows in both treatments were fed the same diet and were subjected to the same environmental and management conditions. Treatments were blind to inseminators.

The main outcome of interest was the PR at the synchronized AI, defined as cows that conceived at the synchronized AI (diagnosed by palpation per rectum between 42 and 48 d after TAI) divided by the total number of cows treated and inseminated.

Progesterone Assay

Expecting a difference in plasma P_4 concentrations of 0.2 ± 0.4 ng/mL at breeding and at 14 d postbreeding (95% confidence and 80% power), 52 cows per treatment were needed (Win Episcopo, 2000). Fifty-five cows per treatment were selected randomly to measure P_4 at TAI and at 14 d later. A blood sample was collected at the time of TAI and 14 d later from the coccygeal vessels using an evacuated blood-collection tube. Samples were centrifuged at $2,500 \times g$ for 30 min and stored at -20°C until analysis was performed. Subsequently, P_4 was determined using a solid-phase, no-extraction radioimmunoassay. The intra- and interassay CV coefficients of variation were 4.8 and 6.1%, respectively. The proportion of cows within treatment having $\text{P}_4 < 1$ ng/mL was calculated at d 0 and 14 after TAI. In addition, the proportion of cows within treatment with different combinations of P_4 at d 0 and 14 was obtained. A concentration of $\text{P}_4 < 1$ ng/mL was considered to be low and $\text{P}_4 \geq 1$ ng/mL was considered to be high. Possible combinations for d 0 and 14, respectively, were high–high, high–low, low–high, and low–low.

Statistical Analysis

A multivariable logistic regression model correcting for potential confounders was conducted to test the main effect of treatment. Explanatory variables (potential confounders) were parity (primiparous vs. multiparous), dystocia (yes vs. no), retained fetal membranes (yes vs. no), breeding season (summer, fall, winter, and

spring) and inseminator. A backward elimination procedure with an inclusion criterion of $P \leq 0.05$ was conducted. Treatment was forced to remain in the final model. Adjusted odds ratios and 95% confidence intervals (CI) were reported. Variables were considered significant at $P \leq 0.05$. Statistical analyses were conducted by using SAS (Version 9.1; SAS Institute, 2003).

The logistic model was defined as follows:

$$\text{Log}(\pi/1 - \pi) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7,$$

where $\pi/1 - \pi$ = odds of the event (conception at synchronized service; yes vs. no); α = intercept; β_1 = parameter of X_1 ; X_1 = effect of treatment; β_2 = parameter of X_2 ; X_2 = effect of parity; β_3 = parameter of X_3 ; X_3 = effect of season; β_4 = parameter of X_4 ; X_4 = effect of inseminator; β_5 = parameter of X_5 ; X_5 = effect of dystocia; β_6 = parameter of X_6 ; X_6 = effect of retained fetal membranes; β_7 = parameter of X_7 ; and X_7 = effect of 2-way interactions.

Statistical analysis of P_4 concentrations was conducted by using a mixed model procedure with cow nested within treatment as a random effect. The model considered parity, breeding season, dystocia, and retained fetal membranes as explanatory variables. Mixed model for P_4 was defined as follows:

$$y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6,$$

where y = progesterone concentrations (at TAI and 14 d later); α = intercept; β_1 = parameter of X_1 ; X_1 = effect of treatment; β_2 = parameter of X_2 ; X_2 = effect of parity; β_3 = parameter of X_3 ; X_3 = effect of season; β_4 = parameter of X_4 ; X_4 = effect of dystocia; β_5 = parameter of X_5 ; X_5 = effect of retained fetal membranes; β_6 = parameter of X_6 ; and X_6 = effect of 2-way interactions.

RESULTS

During 2005, 5,162 cows were subjected to the Presynch protocol. From this number, 3,974 cows were identified in estrus and bred after detected estrus. The remaining 1,188 cows not observed in estrus and not inseminated were assigned randomly to 2 treatments. From the 1,188 cows that started the study, 8 cows from the Pre + Ov + P_4 treatment and 5 cows from the Pre + Ov treatment were not included in the final analysis because of missing data. Therefore, only data from 586 cows from the Pre + Ov + P_4 treatment (203 primiparous and 383 multiparous cows) and 589 cows from the Pre + Ov treatment (203 primiparous and 391 multiparous cows) were considered for the statistical

Figure 1. Pregnancy rate of cows supplemented with progesterone (P_4) differed ($P \leq 0.05$) from control. Control: Ovsynch starting at 73 DIM (Pre + Ov). Progesterone: Ovsynch starting at 73 DIM plus exogenous intravaginal P_4 insert (1.9 g) for 7 d between first GnRH and PGF_{2α} injection of the Ovsynch protocol (Pre + Ov + P_4). Estrous cycles in all cows were presynchronized with 2 doses of PGF_{2α} (Presynch) and only cows not previously inseminated after Presynch injections were assigned to treatments.

analysis. Pregnancy rates which differed ($P \leq 0.05$) between Pre + Ov + P_4 and Pre + Ov treatments, were 31.2 and 22.7%, respectively (Figure 1).

After the backward elimination procedure, there were no significant 2-way interaction terms in the final model. Because parity, breeding season, retained fetal membranes, and inseminator also were not significant variables, they were eliminated during the backward selection process. Only the effects of treatment and dystocia were significant terms and remained in the final model. The adjusted effect of treatment was that cows receiving supplemental P_4 during the Ovsynch were 1.52 times more (95% CI = 1.17 to 1.97) likely to become pregnant than cows from the Pre + Ov treatment. In addition, cows that did not experience dystocia were 1.59 times more (95% CI = 1.05 to 2.42) likely to become pregnant than cows that experienced dystocia at parturition (Table 1).

Concentrations of plasma P_4 are shown in Figure 2. At TAI, P_4 was below 1 ng/mL and did not differ between Pre + Ov + P_4 (0.76 ± 0.3 ng/mL) and Pre + Ov treatments (0.68 ± 0.2 ng/mL). The proportion of cows having $P_4 < 1$ ng/mL (low) was 83.6% (46/55) and 80% (44/55) for Pre + Ov + P_4 and Pre + Ov treatments, respectively. At 14 d after TAI, P_4 was > 1 ng/mL and differed between groups in pregnant and nonpregnant cows. The proportion of cows with $P_4 < 1$ ng/mL was 5.46% (3/55) and 10.9% (6/55) for Pre + Ov + P_4 and Pre + Ov treatments, respectively. In addition, proportions of cows having different combinations of P_4 at d 0 and 14 after TAI for Pre + Ov + P_4 and Pre + Ov treatments were as follows: high-high (14.7 and 12.2%); high-low (0 and 6.1%);

Table 1. Summary of final logistic regression model for pregnancy rate in cows subjected to Ovsynch and treated with progesterone (P_4)

Item	Odds ratio (OR)	95% CI ¹ OR	P-values
Group (P_4 vs. no P_4)	1.52	1.07–1.97	0.0015
Parturition (normal vs. assisted)	1.59	1.05–2.42	0.028

¹CI = Confidence interval.

low–high (80.9 and 75.5%); and low–low (4.4 and 6.1%), respectively. The 2-way interactions, retained fetal membranes, dystocia, or season failed to account significantly for variation in concentrations of P_4 at TAI or 14 d later.

DISCUSSION

The present study was conducted to maximize PR of cows that were subjected to a presynchronization protocol of 2 doses of $PGF_{2\alpha}$ (14 d apart) but not identified in estrus after either of the 2 doses of $PGF_{2\alpha}$. An important aspect of this study was the application of our treatments to a large number of cows that were managed under the same conditions during the entire experimental period. Therefore, a significant source of variation was the use of exogenous P_4 in the Pre + Ov + P_4 treatment.

Figure 2. Least squares means $\pm$ SEM of plasma progesterone (P_4) concentrations in cows with (solid bars) and without (open bars) P_4 supplementation at TAI and at 14 d later within pregnancy status. TAI (d 0) = all cows ($n = 110$); d 14 = all cows 14 d after TAI ($n = 110$); NP d 14 = all nonpregnant cows on d 14 ($n = 77$); and P d 14 = all pregnant cows on d 14 ($n = 33$). Bars having different letters within category differ ($P \leq 0.05$).

Pregnancy rate was significantly greater in cows receiving P_4 than in cows not receiving P_4 . A few studies have investigated similar reproductive protocols and have found comparable results in dairy cattle (Cavestany et al., 2003; El-Zarkouny et al., 2004; Stevenson et al., 2006). Nevertheless, in those 3 studies, cows subjected to the Ovsynch protocol with and without P_4 were not exposed to presynchronizing of estrous cycles (2 doses of $PGF_{2\alpha}$) or, inseminated after estrus before starting the Ovsynch protocol as in the present study. Only Experiment 2 of 1 study (El-Zarkouny et al., 2004) considered a similar protocol similar to the present investigation. Detection of estrus and AI was not part of their protocol during the presynchronization period. Pregnancy rate was improved in cows receiving P_4 only in Experiment 1 of El-Zarkouny et al. (2004) study (45.1 vs. 20.9%). This was dependent on the cyclic status of cows because the improvement of PR was observed only in cows classified as anestrus. In Experiment 2 of the El-Zarkouny et al. (2004) study, the proportion of cows in early diestrus at the onset of the Ovsynch protocol was increased for cows that were subjected previously to the Presynch protocol. In addition, these cows had a greater PR than cows not presynchronized. No interaction between presynchronization and supplementation of P_4 was found; however, results must be interpreted with caution because PR almost doubled in cows that were classified as anestrus, were presynchronized, and received 1.9 g of P_4 compared to similar cows receiving no P_4 (50 vs. 27.3%). Differences were not significant, partly because of lack of power or small sample size to demonstrate statistical differences when cows are subcategorized based on serum P_4 .

Because cows assigned to the current study were subjected to detected estrus and AI but were not identified in estrus during the presynchronization period, this might suggest that a large proportion of cows starting the synchronization program were not cycling. Mean P_4 at TAI was <1 ng/mL and did not differ between treatments. In addition, proportions of cows having P_4 <1 ng/mL did not differ between treatments (83.6 vs. 80% for Pre + Ov + P_4 and Pre + Ov treatments, respectively). This suggests that a large proportion of cows were not in diestrus, and may imply that cows were either adequately synchronized in both treatments, were anestrus, or both.

The proportion of cows having P_4 >1 ng/mL at 14 d after TAI in Pre + Ov + P_4 and Pre + Ov treatment was 94.5 and 89.1% respectively, suggesting that a large proportion of cows in both treatments were cycling; only 4.4 and 6.1% of cows had P_4 <1 ng/mL at TAI and at 14 d later (low–low). These percentages are slightly greater but similar to those reported by Galvão et al. (2004). Nevertheless, our findings indicate that a large

proportion of cows were cycling, although the cycling status of cows at the onset of treatments is unknown. Consequently, based on the present results, we can only suggest that a similar proportion of cows were cyclic after the Ovsynch with or without P_4 . As a result, improved fertility using exogenous P_4 during the Ovsynch protocol might be explained because of greater concentrations of P_4 before TAI determining better synchronization of ovulation than would no supplementation with P_4 (Wehrman et al., 1993; Smith and Stevenson, 1995; Stevenson et al., 2006). In addition, reduced concentrations of $PGF_{2\alpha}$ have been reported for cows during the estrous cycle subsequent to a cycle supplemented with an intravaginal P_4 device, which may be related to better luteal maintenance and improved fertility in dairy cows (Shaham-Albalancy et al., 2001).

Although GnRH without P_4 (Pre + Ov treatment) may result in the release of a preovulatory surge of LH, a small dominant follicle may have limited LH receptors and may contain fewer granulosa cells than a normal, LH-dependent follicle (Macmillan et al., 2003). Consequently, exposure of noncycling cows to P_4 could stimulate the hypothalamic-pituitary axis to trigger mechanisms that facilitate ovulation (Rhodes et al., 2003). In contrast to the present study, Experiment 2 of El-Zarkouny et al. (2004), which compared anestrous cows, based on low P_4 at d -10 and -3 before TAI, that were exposed to 2 presynchronization doses of $PGF_{2\alpha}$, subjected to Ovsynch, and supplemented with ($n = 6$) and without P_4 ($n = 11$), demonstrated no differences in PR (50.0 vs. 27.3%, respectively). Small numbers of cows per treatment, however, were not sufficient for detecting statistical differences.

The greater fertility of cows without than with dystocia reinforces the importance of having a consistent nutrition program during lactation and during the dry period to prevent obese cows and maintain a good transition dairy cow management program to prevent health disorders (NRC, 2001). Cows having dystocia are more likely to develop retained fetal membranes, metritis, and other calving-related disorders (Correa et al., 1993). Consequently, cows experiencing more periparturient diseases have reduced fertility (Gröhn et al., 2003).

In the present study, cows treated with P_4 before TAI had greater concentrations of plasma P_4 14 d after TAI compared with cows subjected to Ovsynch without P_4 supplementation. A normal luteal phase or greater P_4 after AI are consistently associated with better fertility (Thatcher et al., 2002; Spencer et al., 2004; Moore et al., 2005). The fact that Ovsynch protocol supplemented with P_4 during the first 7 d of the synchronization protocol induced greater P_4 after ovulation may indicate that this response resulted from formation of a larger follicle

before ovulation and a larger and a more active corpus luteum after ovulation. Indeed, diameter of the preovulatory follicle 24 h after $PGF_{2\alpha}$ administration has been reported to be greater in cows subjected to Ovsynch and treated with P_4 compared with cows only subjected to the Ovsynch protocol (14.6 ± 0.4 vs. 13.1 ± 0.4 mm, respectively; Stevenson et al., 2004). Nevertheless, in a study using a similar protocol, but with estradiol cypionate instead of GnRH, 24 h after the $PGF_{2\alpha}$ injection and a P_4 insert removal, showed no differences in follicle sizes at ovulation (Galvão et al., 2004). Perhaps another possible explanation for improved fertility and greater P_4 in the Pre + Ov + P_4 treatment than in the Pre + Ov treatment might be that P_4 supplementation may induce more synchronized ovulation and normal luteal phases (Stevenson et al., 2006). Indeed, although the differences were not significant, the proportion of cows having $P_4 < 1$ ng/mL for the Pre + Ov treatment (10.9%) was twice that observed for cows receiving supplemental P_4 (5.5%).

Interestingly, among open cows, treatment with P_4 significantly increased plasma P_4 compared with cows without P_4 . This finding opens a debate regarding P_4 supplementation either before or after AI to improve fertility in dairy cattle. Indeed, it has been reported that high P_4 decreased PR in cows treated either with equine chorionic gonadotropin (Nogueira et al., 2004) or 2 injections of GnRH at d 5 and 15 postbreeding (Bartolome et al., 2005), intended to increase P_4 from the corpus luteum.

CONCLUSIONS

From the results of this study, it was concluded that cows not detected in estrus after presynchronization of estrous cycles by $PGF_{2\alpha}$ and subjected to the Ovsynch protocol starting on d 73 postpartum had greater PR when supplemented with exogenous P_4 during the Ovsynch protocol.

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