Fertility of Lactating Dairy Cows Treated with Ovsynch after Presynchronization Injections of PGF_{2α} and GnRH

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ABSTRACT

Synchronization of ovulation (Ovsynch) using GnRH and PGF_{2α} allows control of follicle growth, corpus luteum regression, and ovulation, but resulting pregnancy rates vary. This study examined whether presynchronization to allow initiation of Ovsynch during diestrus would improve pregnancy rates at timed artificial insemination (AI). Lactating dairy cows ($n = 427$), 69 to 75 d postpartum, were randomly assigned to two groups by parity. Control cows received Ovsynch (GnRH, d 0; PGF_{2α}, d 7; GnRH, d 9; timed AI 16 h after second GnRH). Treated cows received presynchronization injections of PGF_{2α} and GnRH, 10 and 7 d, respectively, before starting Ovsynch. Pregnancy diagnoses were performed 36 d after AI. Progesterone (P₄) concentrations from a subset of cows ($n = 84$) were determined in serum samples collected on d 0, 3, and 7 of Ovsynch. Presynchronization increased the percentages of cows with ≥ 1 ng/ml serum P₄ compared with control cows at first injection of GnRH (d 0; 93 vs. 56%) and on d 3 (90.7 vs. 51.2%) during Ovsynch. On day of PGF_{2α}, d 7 during Ovsynch, percentages of cows with ≥ 1 ng/ml serum P₄ were similar (95.3%, treated vs. 82.9%, control) but more treated cows had ≥ 2 ng/ml serum P₄ (95.3 vs. 63.4%). However, pregnancy to timed AI was similar between treated (41.5%) and control cows (38.3%). Cows with above-average milk production had greater pregnancy rate (45.8 vs. 33.8%) compared with lower producing cows. Although presynchrony increased the proportion of cows with luteal function at onset of Ovsynch, pregnancy rate to timed AI was not improved. Cows with above-average milk production had greater fertility at timed AI than herdmates with lower milk production.

(**Key words:** Ovsynch, presynchronization, fertility)

Abbreviation key: Ovsynch = synchronization of ovulation (GnRH, d 0; PGF_{2α}, d 7; GnRH, d 9; timed

AI 16 h after second GnRH), PR = % pregnant to a timed AI.

INTRODUCTION

Ovsynch [synchronization of ovulation (GnRH, d 0; PGF_{2α}, d 7; GnRH, d 9; timed AI 16 h after second GnRH)] is a management tool that uses GnRH and PGF_{2α} to synchronize ovulation, thus allowing control of first and subsequent timed AI in dairy cows (Pursley et al., 1997a). Pregnancy rate to a timed AI (**PR**) following treatment with Ovsynch is similar to AI after observed estrus (Burke et al., 1996; Pursley et al., 1997a; Britt and Gaska, 1998; de la Sota et al., 1998; Momcilovic et al., 1998; Pursley et al., 1997b, Stevenson et al., 1999). However, studies indicate that rates of synchronized ovulation following Ovsynch range between 80 and 90%. Thus, improvement of synchronization of ovulation following Ovsynch may increase the percentage of cows that become pregnant. Synchronization of ovulation rate is greatest when Ovsynch is initiated midcycle (Vasconcelos et al., 1999). From those data, it appears that if lactating cows were synchronized into early to midluteal stages of the estrous cycle at the start of Ovsynch, pregnancy rates may be increased. This study tested the effect of initiating Ovsynch in cows presynchronized to the early to midluteal phases of the estrous cycle, compared to initiating Ovsynch at random stages of an estrous cycle.

MATERIALS AND METHODS

From February to December 1997, lactating Holstein dairy cows ($n = 427$) from a 700-cow commercial dairy farm in Michigan were randomly assigned to two groups on a weekly basis by parity. The control group received the Ovsynch program that consisted of an intramuscular injection of 100 μ g of GnRH (Cystorelin, Merial Inc., NJ), followed in 7 d with 25 mg of PGF_{2α} (Pharmacia & Upjohn, Kalamazoo, MI), then, 48 hr later, 100 μ g of GnRH. Treated cows received presynchronization injections of 25 mg of PGF_{2α}, 10 d, and 100 μ g of GnRH, 7 d, before initiation of Ovsynch (described above) beginning 49 to 55 d postpartum. All cows received AI 16

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Figure 1. Percentages of cows with ≥ 1 ng/ml progesterone (P_4) in serum during Ovsynch in cows presynchronized with $PGF_{2\alpha}$ and GnRH, 10 and 7 d, before first GnRH of Ovsynch (treated; striped bars) versus Ovsynch only (control; stippled bars).

h following the last injection of GnRH. All cows were 69 to 75 d in lactation at the time of first AI. Laboratory staff administered all injections.

All cows were housed in free stalls, milked twice daily, and received bST beginning at 90 d postpartum. The herd owner/manager performed all AI. The herd veterinarian diagnosed pregnancy at 36 d post AI by rectal examination of the uterus. Progesterone (P_4) concentrations were determined in a subset of cows ($n = 84$) selected randomly within each treatment. Blood samples were collected at the time of the first GnRH (d 0), 3 d after the first GnRH, and at the time of $PGF_{2\alpha}$ (d 7) of Ovsynch. Serum P_4 was quantified by radioimmunoassay (P_4 Coat-a-count kit, Diagnostic Products Cooperation, Los Angeles, CA). Intra- and interassay coefficients of variation were 5.6 and 9.1%, respectively. Daily milk production nearest to time of AI (DHIA milk weight measurements taken every 2 wk) was collected to compare PR in cows above- vs. below-mean milk production. Average daily milk production was 34 kg for first parity, 49.4 kg for second parity, and 49.9 kg for third and greater parities.

Pregnancy rate data were analyzed by chi-square using the Proc GENMOD function of SAS. Covariates considered in the analysis of PR were parity, daily milk production, and interactions of treatment $\times$ parity and daily milk production by parity. Percentages of cows with ≥ 1 ng/ml of serum P_4 or ≥ 2 ng/ml serum P_4 were analyzed by chi-square using the Proc FREQ function of SAS.

RESULTS

Percentages of cows with a functional corpus luteum are summarized in Figure 1. Among treated cows, a

Table 1. Pregnancy rates (%) to timed AI in lactating dairy cows following Ovsynch initiated at a random stage of the estrous cycle (control) or during early to midluteal phase of the estrous cycle (treated).

Parity ¹	Control	Treated	Total ²
First	43.5 (n = 69)	52.8 (n = 73)	48.2 (n = 142)
Second	40.0 (n = 50)	37.9 (n = 58)	38.9 (n = 108)
Third +	33.3 (n = 90)	34.5 (n = 87)	33.9 (n = 177)
Total ³	38.3 (n = 209)	41.5 (N = 218)	39.9 (n = 427)

¹No difference in parity $\times$ treatment ($P = 0.332$).

²First parity different than third+ ($P = 0.008$), others similar ($P > 0.14$).

³No difference in treatments ($P = 0.87$).

greater percentage had ≥ 1 ng/ml serum P_4 compared with control cows ($P = 0.02$) at first injection of GnRH and at 3 d after the first injection of GnRH during Ovsynch. At time of $PGF_{2\alpha}$ (d 7) of Ovsynch, percentages of treated and control cows ≥ 1 ng/ml serum P_4 ($P = 0.35$) were similar. However, in addition to data in Figure 1, the percentage of treated cows with ≥ 2 ng/ml serum P_4 on day of $PGF_{2\alpha}$ was greater than that of control cows (95.3 vs. 63.4%; $P = 0.049$).

There was no effect of treatment on PR ($P = 0.87$; Table 1). However, PR was greater ($P = 0.008$) for first- versus $\geq$ third-parity cows.

Independent of parity, PR of cows with above-mean milk production were greater ($P < 0.025$) compared with cows with below-mean milk production (Table 2). First- and third-plus-parity cows with above-mean milk production had greater ($P < 0.01$) PR compared with herd-mates with milk production below the mean. However, the mean PR for higher producing second lactation cows were similar and not significantly different (Table 2).

DISCUSSION

This study was designed to test the effect of starting Ovsynch in midluteal stage of the estrous cycle on fertil-

Table 2. Pregnancy rates (%) to timed AI of lactating dairy cows following Ovsynch grouped by either above or below mean (μ) milk production within 2 wk of AI.¹

Parity ²	$> \mu$	$< \mu$	Total
First ³	59.7 (n = 67)	37.3 (n = 75)	48.2 (n = 142)
Second	35.6 (n = 55)	43.4 (n = 53)	38.9 (n = 108)
Third + ⁴	42.4 (n = 92)	24.7 (n = 85)	33.9 (n = 177)
Total ⁵	45.8 (n = 214)	33.8 (n = 213)	39.9 (n = 427)

¹DHI data collected every 2 wk; all cows inseminated from 69 to 75 d in lactation.

²Treatment data pooled because treatments did not differ.

³Different within parity ($P < 0.01$).

⁴Different within parity ($P < 0.005$).

⁵Different between groups ($P < 0.025$).

ity in lactating dairy cows. Vasconcelos et al. (1999) observed synchronization rates to Ovsynch of 91% from d 1 to 12 of the estrous cycle compared with 80% from d 13 to 22. Improving synchronization rates by 11 percentage points could increase numbers of pregnancies accordingly.

To synchronize cows in the luteal phase of the estrous cycle, treated cows were injected with PGF_{2α} 10 d, and GnRH 7 d, before start of Ovsynch. Cows responding to PGF_{2α} with luteal regression may ovulate at various times from injection, depending on the stage of development of the ovulatory follicle at that time. Therefore, cows with a dominant follicle in early stages of a follicle wave (<10 mm) at time of PGF_{2α} would likely have a ≥10 mm antral diameter (Pursley et al., 1996) and would likely have LH receptors (Xu et al., 1995) at the time of the subsequent GnRH injection 3 d later, and should respond with ovulation. Cows with a large dominant follicle at time of PGF_{2α} should have either a follicle that would spontaneously cause an LH surge following PGF_{2α}? or respond to a GnRH-induced LH surge 3 d later (Pursley et al. 1996). The treated group was presynchronized so that cows would likely be in the midluteal stage of the estrous cycle at the onset of Ovsynch. The progesterone data are consistent with this intention. In this subset of cows, the percentage of cows with ≥1 ng/ml serum P₄ at the time of first GnRH during Ovsynch and 3 d later was significantly greater in the treated versus control group. The percentage of cows with greater than 1 ng/ml serum P₄ in the control group at first GnRH was similar to a previous study (Pursley et al., 1997b). Treatment also increased the number of cows with more than 2 ng/ml serum P₄ at the time of the final injection of PGF_{2α} during Ovsynch. Theoretically, 66% of the treated cycling cows should be 5 to 9 d postestrus. The other one-third would be 10 to 15 d postestrus. It is likely that 90% of treated cycling cows would be between 5 to 12 d postestrus.

The increase in number of cows in the midluteal phase of the estrous cycle did not affect PR to timed AI following treatment. Pregnancy rates in this study were similar for the treated and control groups. Moreira et al. (2001) presynchronized lactating dairy cows with two injections of PGF_{2α} 14 d apart and 12 d before Ovsynch. Cycling cows had increased fertility due to presynchronization, but when all cows (including anovulatory cows) were considered, there was no effect of presynchronization on fertility. Cartmill et al. (2001) reported greater fertility due to presynchronization in multiparous but not primiparous cows.

The differences reported due to presynchrony on cycling or multiparous cows in the previously mentioned studies may be the result of a positive influence on the uterine environment due to additional occurrences of

estrus before AI. Those studies allowed cycling cows to go through at least one estrus, if not two, whereas in our study, cows were injected 3 d after PGF_{2α} with GnRH. Data from Pursley et al. (1996) indicate approximately one third of cows treated with PGF_{2α} at a random stage of the estrous cycle would have a spontaneous LH surge associated with estrus within the first 72 h following the injection. Thus, it is possible that many cows in our study did not proceed through an additional estrus before Ovsynch. This is likely because an injection of GnRH 72 h following the PGF_{2α} would have prematurely ovulated follicles before a spontaneous estrus and LH surge could occur.

The current data demonstrate that PR decline as parity increases. This trend is consistent with literature reports where synchronization of ovulation was not used (Hillers et al., 1984). However, the effect of parity on PR in this study differs with previous data on synchronization of ovulation (Pursley et al., 1998). In the previous report in which synchronization of ovulation was used, second-parity cows had a higher PR than either first- or ≥ third-parity cows. The disparity could be due to a number of factors related to differences in herd management between the two studies.

An interesting aspect of these data is the effect of milk production on fertility. Cows that had above-mean milk production had greater fertility than cows below the mean. This was a high producing herd, and all cows were near peak milk at time of AI (69 to 75 d in lactation). Previous studies (Spalding et al., 1975; Fonseca et al., 1983; Hillers et al., 1984; Faust et al., 1988) compared fertility of herds at different production levels and found that greater herd milk production negatively affected fertility. There are two possible explanations for the increase in fertility in the cows with above-mean milk production. First, it was likely that cows that produce milk at higher volumes than their herdmates had fewer health problems (feet and legs, mastitis, and periparturient problems such as retained placenta, ketosis, metritis). Badinga et al. (1985) observed that poor-producing cows were less likely to conceive and had a greater number of health-related problems. Secondly, cows with above-average production within a herd are more likely to have double ovulations (Fricke et al., 1998). Cows with multiple ovulations may have a greater chance of conceiving with two or more fertilizable oocytes compared with one.

In summary, presynchronization with PGF?? and GnRH increased the percentage of cows in the midluteal phase of the estrous cycle before starting Ovsynch. However, this did not increase pregnancy rates to timed AI after treatment with Ovsynch. Lactating dairy cows with greater than average milk production also had greater pregnancy rates to timed AI.

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