Short Communication: Presynchronization Using a Single Injection of PGF_{2α} Before Synchronized Ovulation and First Timed Artificial Insemination in Dairy Cows

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ABSTRACT

Conception to synchronized ovulation (Ovsynch) using injections of GnRH and PGF_{2α} and timed artificial insemination has been shown to be maximized when the program is initiated 5 to 12 d after estrus. The objective of this double-blinded field trial was to assess the effect of one injection of PGF_{2α}, 10 d before the Ovsynch program, on the probability of pregnancy at first insemination in lactating dairy cows. The hypothesis was that cows that underwent luteolysis in response to PGF_{2α} would be between 5 and 8 d postestrus at the start of Ovsynch. In five commercial dairy herds in Ontario, Canada, at 52 ± 12 d in milk, 506 cows were assigned at random to receive either one i.m. injection of 500 µg of cloprostenol or saline. Ten days later, all cows received 100 µg of GnRH i.m., followed in 7 d by 500 µg of cloprostenol i.m. and 100 µg of GnRH i.m. 48 h later. All cows were artificially inseminated 0 to 20 h after the second injection of GnRH, without regard to detection of estrus. Pregnancy was diagnosed by transrectal palpation at least 35 d after insemination. The probability of pregnancy after first insemination was modeled with logistic regression, accounting for the correlation of cows with herd and the effect of season of calving. There was no difference in pregnancy risk between cows that received PGF_{2α} presynchronization and controls (37.3 and 36.6%, respectively; odds ratio = 1.03, 95% confidence interval, 0.88 to 1.20). Parity and days in milk at insemination were not significant covariates.

(Key words: presynchronization, Ovsynch, prostaglandin F_{2α}, reproduction)

The Ovsynch protocol for synchronization of ovulation and timed AI in cattle (GnRH followed in 7 d by

PGF_{2α}, GnRH 48 h later, and timed AI 0 to 24 h later; Pursley et al., 1995) represented significant progress in dairy reproductive management by allowing an economically viable probability of pregnancy without detection of estrus. Numerous field trials to evaluate the Ovsynch protocol have been reviewed (LeBlanc, 2001). When started at various stages of the estrous cycle, ovulation after the second injection of GnRH is synchronized in 84 to 87% of cows (Fricke et al., 1998; Vasconcelos et al., 1999). It has been reported (Vasconcelos et al., 1999) that cows that ovulated after the first injection of GnRH were more likely to have a subsequent synchronized ovulation than cows that did not ovulate, and that cows that started Ovsynch on d 5 to 9 after estrus were very likely to ovulate after the first GnRH. In the same study, cows that started Ovsynch on d 5 to 13 had a higher probability of pregnancy and tended to have a lower rate of pregnancy loss than cows started at other stages. Therefore, it appears that Ovsynch is ideally started in early to mid-diestrus. Attempts have been made to use one or two injections of PGF_{2α} before enrollment on Ovsynch (termed presynchronization) to optimize the stage of the estrous cycle for the protocol. Improvements in the probability of pregnancy have been reported following two injections of PGF_{2α} 14 d apart, either 12 d (El-Zarkouny et al., 2001; Moreira et al., 2001) or 14 d (Navanukraw et al., 2002) before beginning Ovsynch. However, in the study by Moreira et al. (2001), among cows that did not receive bST, the benefit of presynchronization was confined to the 77% of cows that were cyclic as determined by plasma P4 > 1 ng/ml at 51 d and/or at 63 d postpartum; when all (non-bST treated) cows were considered, there was no difference in pregnancy risk. In the study by Navanukraw et al. (2002), cows were at various stages of lactation. A single injection of PGF_{2α} 10 or 12 d before Ovsynch has generally failed to increase the probability of pregnancy (Cordoba and Fricke, 2001; Keith et al., 2002; Peters and Pursley, 2002). Cartmill et al. (2001) evaluated one injection of PGF_{2α} 12 d before Ovsynch vs. Ovsynch alone and found an increased probability of pregnancy among multiparous animals at 28 d post-

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AI, which was numerically but not significantly different by 38 to 58 d post-AI. They found no effect of presynchronization in first-lactation cattle at either time. The objective of the present study was to evaluate the ability of one injection of PGF_{2α} 10 d before the start of Ovsynch to improve the probability of pregnancy at first insemination in lactating dairy cows. Our hypothesis was that cows that underwent luteolysis as a result of presynchronization would be between d 5 and 8 after estrus when enrolled on Ovsynch, and that if a sufficient proportion of cows responded to the PGF_{2α}, the overall probability of pregnancy would be improved.

The study population was a convenience sample of five commercial Holstein dairy farms in Southwestern Ontario, Canada, including four free-stall herds (three milking 3×/d) and one tie-stall herd (milked twice/d). All herds fed TMR diets typical of the region, based on alfalfa and corn silage, corn grain, and soybean meal, with various supplements. Herd managers agreed to enroll each lactating cow for her first insemination of the current lactation. Typically, groups of cows were enrolled weekly or biweekly, with a target of insemination between 70 and 77 DIM. Ten days before the start of Ovsynch, cows were assigned randomly within herd to receive PGF_{2α} (500 µg of cloprostenol, 2 ml of Estrumate, Schering-Plough, Pointe Claire, Quebec, Canada) or an equal volume of saline by i.m. injection. Managers were blinded to treatment by providing the experimental injections in individual dose vials identified only with a number, which was recorded for each cow. Ten days later, all cows received the standard Ovsynch protocol (100 µg of GnRH [gonadorelin acetate], Fertiline, Vetoquinol, Lavatrie, Quebec, Canada, followed in 7 d by PGF_{2α}, 100 µg of GnRH 48 h later, all by i.m. injection), and AI without regard to detection of estrus 0 to 20 h after the second GnRH. Herd 1 inseminated cows at the time of the second injection of GnRH, whereas all other herds inseminated cows 14 to 20 h later. Pregnancy was diagnosed by trans-rectal palpation of the uterus at least 35 d after insemination.

The outcome of interest was pregnancy at first insemination, which was modeled with multivariable logistic regression accounting for the correlation of cows within herd with generalized estimating equations (Proc GENMOD in SAS, version 8.0, with binary distribution and compound symmetry correlation structure; SAS Institute, 1999, Cary, NC). In addition to the main effect of presynchronization, covariates including parity (1, 2, or ≥ 3), DIM at AI, and season of calving also were tested. Covariates that were not significant at $P < 0.1$ were removed from the model. Interactions of treatment with each covariate were also tested.

From February 2000 through September 2001, 506 cows completed the experimental protocol. Cows re-

Table 1. Treatment comparisons for pregnancy at first insemination. Lactating Holstein dairy cows in each herd were treated with either one injection of prostaglandin F_{2α}, or with saline, 10 d before starting the Ovsynch protocol for timed insemination.

Herd	n	Treatment		P
		PGF _{2α} Presynchronized + Ovsynch (%)	Control Ovsynch only (%)	
1	145	28.8	23.6	0.48
2	228	46.2	47.8	0.81
3	66	33.3	33.3	1.0
4	23	44.4	35.7	0.99
5	44	20.0	29.1	0.99
Total	506	37.3	36.6	0.87

ceived the experimental injection at a mean of 52 ± 12 DIM, and the median time of AI was 71 DIM. The crude probabilities of pregnancy are listed in Table 1. There was no significant difference in the probability of pregnancy between cows that did and did not receive PGF_{2α} presynchronization (37.3 and 36.6%, respectively, $P = 0.87$). The results of the logistic regression model are shown in Table 2. Although the mean pregnancy risks varied among herds, there was no treatment × herd interaction. Parity and DIM at AI were not significant covariates, but cows that calved in the winter had higher pregnancy risk than those that calved in spring or summer. Accounting for the effect of season, there was no difference between treatments in the probability of pregnancy at first insemination after Ovsynch (odds ratio = 1.03, $P = 0.75$).

The lack of benefit of presynchronization in the present study may be attributable to lack of luteolytic response to the “set-up” injection of PGF_{2α} and subsequent estrus. Alternatively, if cows did undergo luteolysis but were not in estrus until 5 to 7 d later, they would be too early in the subsequent cycle for optimal response to Ovsynch. Additionally, the corpus luteum of cyclic cows that were on d 3 to 5 of the estrous cycle at the initial PGF_{2α} would not be responsive. Therefore, such cows would start Ovsynch between d 13 to 15 of the cycle, and would likely fail to be synchronized because of spontaneous luteolysis and ovulation before the end of Ovsynch. Unfortunately, samples of blood or milk were not collected for progesterone analysis to assess the cyclicity of cows at the start of the protocol, or to document luteolysis. In a group of cyclic cows distributed throughout the estrous cycle, on a given day approximately half would be expected to be in diestrus with a corpus luteum responsive to PGF_{2α} (d 7 to 17 of the cycle). Given the lack of effect of presynchronization, we speculate that substantially fewer than half of the cows that received PGF_{2α} underwent luteolysis, and therefore were no more optimized as to stage of the

Table 2. Logistic regression model of the probability of pregnancy at first insemination in 506 lactating Holstein dairy cows treated with one injection of prostaglandin F_{2α}, or saline, 10 d before starting the Ovsynch protocol for timed insemination.¹

Variable	Odds ratio	95% Confidence interval	P
PGF _{2α} presynchronization	1.03	0.88 to 1.20	0.75
Month of calving			
March – May	0.55	0.40 to 0.76	0.001
June – August	0.76	0.64 to 0.90	0.002
September – November	0.61	0.27 to 2.15	0.24
December – February	referent	...	...

¹Adjusted for the correlation of cows within herd with generalized estimating equations.

estrous cycle at the start of Ovsynch than the controls. In addition, there is evidence from different management and climatic conditions that approximately 20% of lactating dairy cows may be anovulatory at 50 to 70 DIM (Opsomer et al., 2000; Cartmill et al., 2001; Moreira et al., 2001; Stevenson, 2001; Gumen et al., 2002). It is possible that a minority of presynchronized cows did have an increased pregnancy risk, but that effect was counterbalanced by a subset of cows in which presynchronization at –10 d resulted in starting Ovsynch on d 3 or 4 of the subsequent estrous cycle, which would reduce pregnancy risk (Vasconcelos et al., 1999).

In conclusion, the present results are consistent with a lack of overall increase in the probability of pregnancy at first insemination after presynchronization with one injection of PGF_{2α} 10 d (Keith et al., 2002; Peters and Pursley, 2002) or 12 d (Cordoba and Fricke, 2001) before Ovsynch, or with two injections of PGF_{2α} 14 d apart, 12 d before Ovsynch (in the absence of bST; Moreira et al., 2001). Reports of increases of approximately 10 percentage points in the probability of pregnancy after presynchronization with two injections of PGF_{2α} were either restricted to cows known to be cyclic (Moreira et al., 2001), or in study populations that apparently were not restricted to first inseminations <100 DIM (Nananukraw et al., 2002). Collectively, currently available published results suggest potential benefits of presynchronization in cyclic and multiparous cows, but a need for caution in whole-herd implementation of presynchronization for first insemination.

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