Short Communication: Conception Rates Following Detection of Estrus and Timed AI in Dairy Cows Synchronized Using GnRH and PGF_{2α}

J. C. Dalton,¹ R. Manzo,¹ A. Ahmadzadeh,¹ B. Shafii,² W. J. Price,² and J. M. DeJarnette³

¹Animal and Veterinary Science Department, and

²Statistical Programs, College of Agricultural and Life Sciences, University of Idaho, Moscow 83844

³Select Sires, Inc., Plain City, OH 43064

ABSTRACT

The objective of this study was to compare conception rates of cows exhibiting spontaneous estrus and receiving artificial insemination (AI) before completion of a timed AI protocol with cows that did not display estrus spontaneously, but were inseminated after 1 of 3 GnRH-PGF_{2α} protocols. Cows ($n = 432$) in 2 herds were administered GnRH on d -7 and were tail-chalked daily. Cows detected in estrus before d 0 were inseminated immediately. Cows not detected in estrus by d 0 were administered PGF_{2α} and were tail-chalked daily until 48 h after PGF_{2α}. Cows detected in estrus from d -7 to 48 h after PGF_{2α} were inseminated and designated as treatment A ($n = 46$). Cows not detected in estrus and not inseminated by 48 h after PGF_{2α} were assigned randomly to receive either GnRH 48 h after PGF_{2α} and timed AI 16 h later (treatment B; $n = 132$), or GnRH and timed AI 64 h after PGF_{2α} (treatment C; $n = 127$), or timed AI 64 h after PGF_{2α} (treatment D; $n = 127$). Pregnancy was diagnosed 38 to 45 d after AI by palpation per rectum of uterine contents. Nearly 11% of all cattle exhibited spontaneous estrus and received immediate AI. Herd did not influence the percentage of cows detected in estrus and inseminated. Conception rates did not differ among treatments. Conception rates differed between herds, but no interaction of herd $\times$ treatment was detected. No differences were detected between herds for days in milk, milk production, AI service number, or parity.

(Key words: conception rate, detection of estrus, synchronization, timed AI)

Ovsynch (GnRH-7 d-PGF_{2α}-2 d-GnRH-0 to 24 h-timed AI; Pursley et al., 1995, 1997) was designed to synchronize ovulation; thereby allowing timed AI of all cows without detection of estrus. The ability of Ovsynch to synchronize ovulation, however, is dependent upon

the stage of follicular development at the time of the initial GnRH injection (Vasconcelos et al., 1999). Consequently, presynchronization of estrous cycles (Presynch) by using 2 injections of PGF_{2α} 14 d apart, with the second injection either 12 (Moreira et al., 2001; El-Zarkouny et al., 2004) or 14 d (Navanukraw et al., 2004) before the initiation of Ovsynch has been shown to increase conception rates by optimizing the stage of the estrous cycle (between d 5 and 13) at initiation of Ovsynch. Nevertheless, presynchronization is a time- and labor-intensive protocol that is most effective in scheduling first services. Presynchronization was not designed nor is it practical for application to previously inseminated cows that failed to conceive. Ovsynch, without presynchronization, remains an effective breeding tool, especially for second and greater AI services.

Fricke et al. (1998) observed an 84% incidence of ovulation after the second injection of GnRH when Ovsynch was initiated at various stages of the estrous cycle. Vasconcelos et al. (1999) reported that ovulation to the second GnRH ranged from 81 to 94% with an overall average synchronization rate of 87%. Vasconcelos et al. (1999) stated that initiation of Ovsynch in cows in the second half of the estrous cycle resulted in a lower synchronization rate, with failures primarily due to ovulation before the second GnRH injection. Furthermore, DeJarnette et al. (2001) reported a 20% incidence of spontaneous estrus (before the second injection of GnRH) in lactating dairy cows enrolled in Ovsynch. Consequently, conception rates of cows enrolled in Ovsynch, and displaying spontaneous estrus and (or) spontaneous ovulation before completion of the protocol, would likely be compromised when inseminated at a fixed time.

Pursley et al. (1998) investigated the optimal time for AI after the second GnRH injection of the Ovsynch protocol and reported that lactating dairy cows inseminated at 0, 8, 16, and 24 h after GnRH had similar conception rates, whereas those inseminated 32 h after GnRH had reduced conception rates. Nevertheless, conception rates at 0 to 32 h produced a quadratic response curve, with the greatest numerical value found for cows

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Corresponding author: Joseph C. Dalton; e-mail: jdalton@uidaho.edu.

Figure 1. All cows were treated with GnRH on d -7. Routine daily tail chalk application and detection of estrus was conducted from d -7 to 0. Cows detected in estrus before d 0 were inseminated, designated as treatment A, and removed from the remainder of the protocol. Cows not detected in estrus by d 0 received PGF_{2α} and continued to receive daily tail chalk for 48 h. All cows detected in estrus up to 48 h after PGF_{2α} received AI and also were designated as treatment A. Cows not detected in estrus and not inseminated by 48 h were assigned randomly to 1 of 3 treatments: GnRH 48 h after PGF_{2α} and timed AI 16 h later (treatment B); GnRH and timed AI 64 h after PGF_{2α} (treatment C); or timed AI 64 h after PGF_{2α} (treatment D).

inseminated at 16 h after GnRH (Pursley et al., 1998). Given that: 1) Ovsynch (even without presynchronization) remains an effective breeding tool, and 2) spontaneous estrus may occur in up to 20% of cattle enrolled in Ovsynch without the benefit of presynchronization (DeJarnette et al., 2001), the objective of this study was to compare conception rates of cows exhibiting spontaneous estrus and receiving AI before completion of a timed AI protocol with those that did not display estrus spontaneously, but were inseminated after 1 of 3 GnRH-PGF_{2α}-based timed AI protocols.

Two commercial herds located in Idaho and Utah participated in the study. Cattle were housed in combination dry-lot, free-stall housing on both dairies. Average yearly milk yield (and herd size) was 10,454 kg per cow (500 cows) and 11,500 kg per cow (975 cows) for the Idaho and Utah herds, respectively. Cattle were fed a TMR formulated to meet or exceed requirements for lactating dairy cows (NRC, 2001). Cattle were restrained in headlocks once daily to facilitate chalking tails, detection of estrus based on rubbed tail chalk (roughened tailhead hair), and AI. Semen used for AI was from multiple suppliers.

Cows (n = 432) in 2 herds diagnosed as not pregnant to a previous AI and cows >60 d postpartum (and not yet inseminated) were treated with GnRH (100 µg; Cystorelin, Merial, Athens, GA) on d -7 (Figure 1). Routine tail chalking and detection of estrus was conducted from d -7 to 0. Cows detected in estrus according to chalk removal (roughened tailhead hair) before d 0 were inseminated immediately. Cows not detected in estrus by d 0 were administered PGF_{2α} (25 mg; Lutalyse, Pfizer, New York, NY) and continued to receive daily tail chalk until 48 h after PGF_{2α}. All cows detected

in estrus from d -7 to 48 h after PGF_{2α} were inseminated immediately and were designated as treatment A (n = 46). Cows that were not detected in estrus and not inseminated by 48 h after PGF_{2α} were assigned randomly to 1 of 3 treatments: 1) GnRH (100 µg) 48 h after PGF_{2α} and timed AI 16 h later (treatment B; n = 132); 2) GnRH (100 µg) and timed AI 64 h after PGF_{2α} (treatment C; n = 127), or 3) timed AI 64 h after PGF_{2α} without GnRH (treatment D; n = 127) (Figure 1). Pregnancy was diagnosed 38 to 45 d after AI by palpation per rectum of uterine contents.

Conception rates were analyzed using a logistic regression model including the effects of herd, treatment, and 2-way treatment interactions, in addition to DIM, milk production, AI service number, and parity. The required sample size to achieve a 10% depression in conception rate is 415 dairy cows, which corresponded to a 5% level of significance, with a power of 70% (Hsieh et al., 1998). Days in milk, milk production, AI service number, and parity were compared between herds using the GLM procedure in SAS (SAS Institute, 2000). All computations were carried out using SAS (2000).

Nearly 11% of all cows (46/432) enrolled exhibited spontaneous estrus, were inseminated, and removed from the remainder of the protocol (treatment A). The percentage of cows detected in estrus and inseminated (treatment A) did not differ between herds. Conception rates did not differ among treatments (Table 1). Further, with 432 observations and an overall conception rate of 24.7%, at least 70% power is achieved for the detection of odds ratios less than 0.5 or greater than 2.0 with a significance level equal to 5%. These odds ratios translate to a change in the probability of conception to less than 15% or greater than 40% (Hsieh et al.,

Table 1. Effect of AI protocol on conception rates (% with no. pregnant/no. inseminated in parentheses) of lactating dairy cows.

Herd	Treatment ¹				Total
	A	B	C	D	
A	32.1 (9/28)	33.3 (30/90)	29.2 (26/89)	21.2 (18/85)	28.4 ^a (83/292)
B	16.7 (3/18)	26.2 (11/42)	13.2 (5/38)	11.9 (5/42)	17.1 ^b (24/140)

^{a,b}Herd influenced ($P < 0.05$) overall conception rate across treatments, but no herd $\times$ treatment interaction was detected.

¹Treatment A = detection of estrus and AI before 48 h after PGF_{2 α} ; treatment B = injection of GnRH 48 h after PGF_{2 α} and AI 16 h later; treatment C = injection of GnRH and AI 64 h after PGF_{2 α} ; and treatment D = insemination at 64 h after PGF_{2 α} .

1998). Nevertheless, even with 70% power, it is possible that treatment D resulted in a lower conception rate, but the statistical power of this study was not adequate to detect this difference.

Conception rates differed ($P < 0.05$) between herds (28.4 vs. 17.1%), and no interaction of herd $\times$ treatment was detected (Table 1). Further, no difference was detected between herds for DIM, milk production, AI service number, or parity.

Using milk progesterone analyses, Reimers et al. (1985) reported the proportion of cows not in or near estrus when inseminated varied from 0 to 60% among dairy herds. Phatak and Touchberry (1988) reported that the accuracy of tail chalk as an estrus-detection aid varied from 38 to 50%, based on milk progesterone concentration or subsequent pregnancy after AI. Clearly, the misdiagnosis of estrus contributes to poor reproductive performance. Nevertheless, no differences were found between herds in the percentages of cows detected in estrus and inseminated. Therefore, it is unlikely that the difference observed in average conception rate between the herds is due to inaccurate detection of estrus.

Although Pursley et al. (1998) reported similar conception rates for lactating dairy cows inseminated at 0, 8, 16, and 24 h after the second GnRH injection of the Ovsynch protocol, and those inseminated 32 h after GnRH had reduced conception rates, conception rates of cows inseminated at 0 to 32 h after GnRH produced a quadratic response curve, which peaked at 16 h. In practice, timed AI 16 h after the second GnRH is inconvenient because it necessitates handling cattle 4 times, with timed AI occurring opposite (a.m. or p.m.) to the previous 3 handlings. Treatment C (GnRH and timed AI 64 h after PGF_{2 α}) is a variation of Co-Synch (Geary and Whittier, 1998) and was developed in an effort to reduce animal handling and optimize conception rates. The response time from PGF_{2 α} to estrus has been reported to be, on average, between 64 and 73 h (Walker et al., 1996; Dalton et al., 2001). Although a reduction in animal handling occurred, no conception rate advan-

tage was detected when administering GnRH and timed AI together 64 h after PGF_{2 α} .

In another study investigating variations of Ovsynch (without presynchronization), GnRH and timed AI 72 h after PGF_{2 α} appeared to be without benefit compared with GnRH and timed AI 48 h after PGF_{2 α} or GnRH 48 h after PGF_{2 α} and timed AI 24 h later (conception rates = 19.1, 24.3, and 27.1%, respectively; M. A. Portaluppi and J. S. Stevenson, 2005, Kansas State Univ., Manhattan, unpublished data).

In contrast, Portaluppi and Stevenson (2005) reported in another study investigating variations of Ovsynch, that administering GnRH and first-service timed AI 72 h after PGF_{2 α} produced greater conception rates (in cows whose estrous cycles were presynchronized) than the other 2 treatments (GnRH and timed AI 48 h after PGF_{2 α} , and GnRH 48 h after PGF_{2 α} and timed AI 24 h later) combined. Taken together, the optimal time for AI in the Ovsynch protocol may be different depending upon whether presynchronization is used.

In an effort to exploit the average response time from PGF_{2 α} to estrus, and achieve timed AI without the costly administration of a second GnRH injection, treatment D was developed. Timed AI (without administration of GnRH) 64 h after PGF_{2 α} does not seem to be advantageous. Although the present study had a power of 70% ($\alpha = 0.05$, $\beta = 30\%$), the probability of a type II error must be considered, as the likelihood of detecting treatment effects may be limited by the number of cows enrolled in the study. In a study utilizing modified targeted breeding (PGF_{2 α} 14 d before GnRH, followed 7 d later by PGF_{2 α} , detection of estrus and AI, with cows not observed in estrus receiving timed AI at 72 h), Jordan et al. (2002) suggested that fertility was reduced in cattle not observed in estrus and receiving timed AI (without administration of GnRH) 72 h after PGF_{2 α} .

Although addition of a controlled internal drug-releasing intravaginal insert containing progesterone into the Ovsynch protocol may solve the problem of precocious spontaneous estrus and ovulation, it adds

significantly to the cost (\$9.00) of the protocol. In contrast, detection of estrus, especially in the form of daily application and reading of tail chalk, is a routine, daily procedure on many farms for all breeding-eligible cattle. Therefore, inclusion of detection of estrus in the Ovsynch protocol may add little, if any, cost to the protocol. Furthermore, a cost savings may actually accrue by using limited detection of estrus, immediate AI, and removal of animals from a protocol because scheduled GnRH and (or) PGF_{2α} injections could be eliminated depending upon when a cow came into estrus and received AI (DeJarnette et al., 2001).

In conclusion, although the percentage of cows detected in estrus ≤48 h after PGF_{2α} in the present study (11%; 46/432) was less than previously reported (20%; 68/345; DeJarnette et al., 2001), dairy producers should consider carefully the use of Ovsynch without detection of estrus in dairy cows in which estrous cycles are not presynchronized. Cows enrolled in Ovsynch and exhibiting spontaneous estrus before timed AI may not become pregnant because the timed AI occurs after estrus and ovulation. In this scenario, if fertilization occurs, fertilization of an aging ovum would be likely, and reduced embryo quality and reduced conception rates would be expected (Dalton et al., 2001). Lastly, treatment C (GnRH and timed AI 64 h after PGF_{2α}) and treatment D (timed AI 64 h after PGF_{2α} without administration of GnRH) were unsuccessful in increasing the conception rate.

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