INSEMINATIONS AT ESTRUS INDUCED BY THE PRESYNCH PROTOCOL BEFORE TIMED ARTIFICATION INSEMINATION

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Summary

A controlled field study examined conception rates after two timed-AI (TAI) breeding protocols conducted on two commercial dairy farms. Estrous cycles in postpartum lactating cows were presynchronized with two injections of $PGF_{2\alpha}$ given 14 days apart (Presynch) and then, after 12 days, the standard Ovsynch protocol (injection of GnRH 7 days before and 48 h after an injection of $PGF_{2\alpha}$, with one TAI at 12 to 16 hours after the second GnRH injection) or Heatsynch protocol (injection of GnRH 7 days before an injection of $PGF_{2\alpha}$, followed 24 h later by 1 mg of estradiol cypionate (ECP) and one TAI 48 hours after ECP) was applied. Experimental design allowed for AI to occur any time after the second Presynch injection and during the designed breeding week when estrus was detected. Of the 1,846 first services performed, only 1,503 (rate of compliance = 81.4%) were performed according to protocol. Numbers of cows inseminated, logistic-regression-adjusted conception rates, and days in milk (DIM) were for inseminations made: 1) during 14 days after first Presynch injection (n = 145; 22.6%; 54 ± 0.4 DIM); 2) during 12 days after second Presynch injection (n = 727; 33%; 59 ± 0.2 DIM); 3) during 7 days after the first GnRH injection of Ovsynch or Heatsynch (n = 96; 32.1%; 74 ± 0.5 DIM); 4) after estrus as part of Heatsynch (n = 212; 44.6%; 76 ± 0.3 DIM); 5) after TAI as part of Heatsynch (n = 154; 21.1%; 76 ± 0.4 DIM); 6) after estrus as part of Ovsynch (n = 43; 48.7%; 77 ± 0.7 DIM); and 7) after TAI as part of Ovsynch (n = 271; 24.4%; 77 ± 0.3 DIM). Conception rates when AI occurred after one Presynch injection were less than when AI occurred after two Presynch injections. Conception rates for those inseminated after either Presynch injection did not differ from those inseminated after combined Heatsynch + Ovsynch. Cows in the Ovsynch and Heatsynch protocols inseminated after estrus during the breeding week had greater conceptions rates than those receiving the TAI, but overall conception rates did not differ between protocols. Among cows inseminated after detected estrus, conception was greater for cows in the Heatsynch + Ovsynch protocol (77 ± 0.4 DIM) than for those inseminated after either Presynch injection (54 ± 0.4 or 59 ± 0.2 DIM). We concluded that conception rates after Heatsynch and Ovsynch were similar under these experimental conditions, and that delaying first AI improved fertility for cows inseminated after detected estrus.

(Key Words: Calving Difficulty, Compliance, Conception, Synchronized Estrus.)

Introduction

Various programmed-breeding protocols have been developed to synchronize estrus and ovulation in lactating dairy cows to initiate first services. These include the standard Ovsynch protocol and variations that may include estrogen administration to induce estrus during the breeding week (Heatsynch). The only estrogen product in the United States (estradiol cypionate; ECP) was recently removed from the market. In general, conception or pregnancy rates after either of these two protocols are similar. When estrous cycles are presynchronized, or staged according to days of the estrous cycle, so that a majority of cows are in mid diestrus (d 5 to 12) at the onset of the Ovsynch protocol, resulting pregnancy rates are enhanced, compared with those of cows beginning the Ovsynch protocol at random stages of the estrous cycle.

A question often asked is whether it is advisable to inseminate cows earlier during the administration of these protocols when estrus is detected after either of the Presynch injections of $PGF_{2\alpha}$ and the cow is at or near the end of the voluntary waiting period (VWP). Conception rates for cows inseminated after estrus tend to be greater or similar to those of cows receiving timed AI (TAI) to which are applied various Ovsynch-like protocols.

A related question is what is the ideal VWP to be applied on individual dairy farms or in the industry today, in which most cows are inseminated after some controlled breeding program. Earlier studies (before application of controlled-breeding programs) in which cows were submitted for AI at predetermined DIM generally reported a significant or numerical trend for increased conception after a longer VWP. A recent study was conducted in which low- and high-producing dairy cows were inseminated at different DIM as part of the Ovsynch protocol. In both milk-production groups, conception rates were improved by delaying first services 3 weeks, from 53 to 59 (14.4%) to 73 to 81 DIM (34.5%) for low-producing cows and from 73 to 81 (28.2%) to 94 to 102 DIM (41.4%) for high-producing cows.

The objective of our study was to determine pregnancy outcomes of two standard breeding protocols (Ovsynch and Heatsynch) in which estrous cycles were presynchronized previously with two injections of $PGF_{2\alpha}$ (Presynch), and inseminations occurred whenever estrus was detected any time after the second Presynch $PGF_{2\alpha}$ injection. Ancillary objectives were to determine compliance to the designed protocols and whether DIM was a significant factor in accounting for different conception rates.

Experimental Procedures

A controlled study was conducted at two (Dairy #3 and Dairy #5) of five Foster Dairy Farms, Hickman, California. Lactating Holstein cows (n = 1,846) that calved between February 2, 2001, and February 1, 2002, were included in the study, which consisted of first postpartum inseminations conducted between March 22, 2001 and April 28, 2002. All five Foster Dairy Farm herds were managed similarly by one central management team, having different herdsmen and inseminators at each dairy. All cows at each dairy were fed a total mixed ration (TMR) to meet or exceed requirements recommended for lactating dairy cows. Diets were mixed from common ingredients located at Dairy #2 to feed cows at Dairy #2 and #3 or at Dairy #5 to feed cows at Dairy #4, #5, and #6. Diets consisted of alfalfa, soybean meal, bypass soya, corn silage, barley, flaked corn, brewer's grains, beet pulp, and added minerals. Cows were milked and fed three times dailym so fresh feed was available when cows returned to pens from the milking parlor after each milking (0400, 1200, and 2000 h). No recombinant bST was used in these herds.

The study was designed to compare conception rates of lactating dairy cows inseminated at first service in response to one of two estrus- and ovulation-synchronization protocols (Ovsynch [injections of GnRH 7 days before and 48 hours after $PGF_{2\alpha}$] and Heatsynch [injection of GnRH 7 days before and an injection of ECP 24 hours after $PGF_{2\alpha}$]). Before applying each protocol, estrous cycles of cows were presynchronized by using two injections of $PGF_{2\alpha}$ (13 to 15 days apart; Presynch), with the second Presynch injection occurring 11 to 12 days before initiating either of the two protocols. Cows were assigned to begin the Presynch injection sequence on the basis of calving dates, grouped into breeding clusters every 10 days, beginning no sooner than 40 DIM. Doses and sources of hormones were: $PGF_{2\alpha}$ (25 mg; Lutalyse, Pharmacia Animal Health, Kalamazoo, MI); estradiol cypionate (1 mg; ECP; Pharmacia Animal Health, Kalamazoo, MI), and GnRH (100 µg; Cystorelin, Merial Limited, Iselin, NJ). All hormones were administered i.m. in the gluteal muscles.

Experimental protocols were designed for insemination of cows after detected estrus that occurred: 1) during 12 days after the second of two Presynch injections; 2) during 7 days after the first GnRH injection of Ovsynch or Heatsynch (some received $PGF_{2\alpha}$ 7 days later and were inseminated that day); or 3) during the breeding week when the ECP injection of Heatsynch or the second GnRH injection of Ovsynch was administered. Therefore, any cow that was detected in estrus 24 or more hours after the $PGF_{2\alpha}$ injection of the Ovsynch or Heatsynch protocol was eligible for insemination by design. In the absence of previous AI, cows in the Ovsynch protocol were inseminated at 12 to 16 hours after the second GnRH injection and those in the Heatsynch protocol were inseminated at 48 hours after ECP injection.

All hormonal injections and their dates of administration, calving difficulty scores (CDS), calving and breeding dates, AI at estrus or TAI, pregnancy outcomes, etc., were recorded in DHI records (DHI-Provo). As a result, actual dates of hormonal injections and inseminations could be verified to determine protocol compliance. Of the 1,846 inseminations recorded during the experimental period, some cows were inseminated contrary to study design.

Four individuals conducted all inseminations at Dairy #3 and four different individuals conducted inseminations at Dairy #5. The same AI sires were used at both dairies. Therefore, effects of inseminator were confounded with herd. Inseminations associated with estrus were conducted between 8 and 16 hours after detected estrus (a.m. - p.m. rule). Detected estrus was defined to include: 1) visually detected standing to be mounted, 2) tail-chalk rubs, and 3) other secondary signs (mucus or ruffled tailhead hair). Where chalk rubs or secondary signs were detected, cows were often palpated to detect uterine tone for validation of potential accuracy of the suspected estrus. These determinations of defined estrus were judgments made by the inseminators.

Cows were locked up at the feed bunk each morning between 8 and 10 a.m. to conduct hormonal injections, read and apply new tail chalk, check for pregnancy, and perform other health treatments. When cows were detected in standing estrus after morning chalk reads, they were inseminated that evening.

Conception rates (no. of pregnancies ÷ no. of cows inseminated) were calculated from pregnancy checks that were conducted weekly by palpation per rectum of the uterus and its contents at a minimum of 35 to 41 days after last AI.

Results and Discussion

Of the 1,846 first services performed, only 1,503 (81.4%) were performed according to the designed protocol. The breaches in protocol included missed or mistimed injections, hormone-injection sequence errors, and, in some instances, cows receiving both ECP and GnRH during the breeding week. Our protocol dictated that cows were eligible to be inseminated any time once estrus was detected after the second Presynch injection until the scheduled TAI associated with the Heatsynch or Ovsynch protocols. Noncompliant inseminations included those conducted during 14 days after the first Presynch injection (n = 145; 7.9%) and those (n = 198; 10.7%) that did not comply with the synchronization protocol. Of the two deviations from insemination protocol, the latter was most serious because pregnancy outcomes were most compromised, as measured by actual conceptions rates (22.2%), which included inseminations after detected estrus (conception rate = 26%; n = 130) and TAI (conception rate = 15%; n = 68).

Inseminations made after the first Presynch injection were included in statistical analyses so conception rates after fewer DIM could be examined. Of 1,648 inseminations performed after the five breeding scenarios, 968 (58.7%) occurred after either one (1×PGF) or two (2×PGF) injections of $PGF_{2\alpha}$, or during 7 days after the beginning GnRH injection of Heatsynch or Ovsynch. More (P = 0.01) cows in the Heatsynch protocol were inseminated during the designed breeding week, after or in association with expressed estrus (57.9%), than in the Ovsynch protocol (13.6%). These differences between Ovsynch and Heatsynch were consistent across lactation numbers, but more first- and second-lactation cows than older cows were detected in estrus after Heatsynch, compared with those after Ovsynch (treatment × lactation number interaction; $P < 0.01$; first lactation: 63.6 vs. 17.8%; second lactation: 67.8 vs. 9.8%; and third or greater lactation: 47.8 vs. 13.6%, respectively).

Days in milk at each insemination, on the basis of the seven statistically compared protocols, are summarized in Table 1. Cows in 1×PGF were inseminated 5 ± 0.2 d earlier (P<0.01) than were cows in 2×PGF, whereas both groups were inseminated 15 to 20 d earlier (P<0.01) than cows inseminated during 7 days after the beginning GnRH injection of Heatsynch or Ovsynch, and 17 to 23 days earlier (P<0.01) than those that completed the designed Heatsynch and Ovsynch protocols.

Logistic-regression-adjusted mean conception rates are summarized in Table 1 for the five breeding scenarios, as are orthogonal contrasts of adjusted means. Although significant differences were detected for three of six contrasts, some contrasts included confounding treatment protocols with DIM.

The first contrast indicated that the adjusted conception rates increased ($P < 0.05$) from 22.6 to 33% because of the additional injection of $PGF_{2\alpha}$, but DIM at AI also increased ($P < 0.01$) from 54 to 59 (Table 1). Therefore, it is not clear whether the difference in conception occurred because of the additional $PGF_{2\alpha}$ injection or because DIM was longer at AI.

The second contrast compared cows receiving both Presynch injections with those inseminated during 7 days after receiving the beginning GnRH injection of the Heatsynch or Ovsynch protocol. The latter scenario also included some cows that received the $PGF_{2\alpha}$ injection for those two protocols, but were inseminated on that day before receiving subsequent injections associated with Heatsynch (i.e., ECP) or Ovsynch (i.e., GnRH). These cows were likely coming into estrus spontaneously despite the injection of $PGF_{2\alpha}$. Although DIM was longer ($P < 0.01$) for cows in the latter protocol, conception rates (33 vs. 32.1%) did not differ from the 2×PGF cows (Table 1). Again, this contrast confounds treatment (two $PGF_{2\alpha}$ injections vs. two or three $PGF_{2\alpha}$ injections + GnRH) with DIM.

The third contrast also confounds DIM with treatment protocol (detected estrus vs. detected estrus + TAI). Although all cows were inseminated after detected estrus in 1×PGF, 2×PGF, and 2×PGF + GnRH ± PGF groups, only 37.5% of the cows in Heatsynch + Ovsynch were inseminated after, or in association with, expressed estrus. Conception rates (28.3 vs. 31.5%) did not differ between groups (Table 1).

The fourth contrast (TAI: Heatsynch + Ovsynch vs. estrus: Heatsynch + Ovsynch) compared conception rates of cows inseminated after TAI vs. after detected estrus. When treated with either the Heatsynch or Ovsynch protocol, cows inseminated after, expressed estrus had greater (P<0.001) conception rates (45.3 vs. 23.2%) than those receiving the TAI at similar DIM (Table 1),

The fifth contrast was a comparison of the overall outcomes from the Heatsynch vs. Ovsynch protocols (Table 1). Pregnancy outcomes did not differ between the two protocols. Conception rates of cows inseminated after Heatsynch and Ovsynch, when estrus occurred, were nearly identical, as were conception rates for cows receiving the TAI regardless of protocol (Table 1). Overall numerical differences between Heatsynch (34.7%) and Ovsynch (27.7%) are explained by proportionally more cows having greater conception rates after inseminations associated with expressed estrus (Heatsynch vs. Ovsynch: 57.9 vs. 13.6%), compared with fewer cows having lower conception rates after receiving the TAI.

The sixth contrast compared conception rates of cows after detected estrus at different DIM (Estrus: 1×PGF + 2×PGF vs. estrus: Heatsynch + Ovsynch). This contrast verified that conception rates were increased (P<0.01) when inseminations, based solely on estrus, occurred after more DIM (31.3 vs. 45.3%; Table 1).

One might argue that the additional hormonal treatments (GnRH, $PGF_{2\alpha}$, and ECP [Heatsynch cows only]) applied to cows in the Heatsynch and Ovsynch protocols could account for observed differences. This was not true, however, in a recent study in which low- and high-producing dairy cows were inseminated after an Ovsynch protocol applied at different DIM. In both milk-production groups, conception rates were improved by delaying first services 3 weeks, from 53 to 59 DIM (14.4%) to 73 to 81 DIM (34.5%) for low milk-producing cows and from 73 to 81 DIM (28.2%) to 94 to 102 (41.4%) for high-producing cows.

A large percentage of the cows calved unassisted (CDS = 1; 1,282 of 1,846; 69.4%). Numbers of cows with scores of 2 (14%), 3 (14.8%), 4 (2%), and 5 (0.3%) made up the remaining proportions. Greater CDS was associated negatively with subsequent conception rate. For every 1-unit (range of 1 to 5) increase in calving difficulty, conception rate was reduced (P<0.01) by 4.8 ± 1.4%. Those having a CDS of 1 or 2 had greater (P<0.05) conception rates than those having a CDS of 3 or more (33.8 vs. 24.6%). Poorer fertility of cows having calving difficulty, because of its associated uterine pathology, is consistent with other reports.

Herd had no effect on pregnancy outcomes. All of the probability values for herd were > 0.4. This is attributed to both herds being fed the same TMR from a common feed supply, being managed under similar policies, and using common AI sires, even though different people detected estrus and performed inseminations at each dairy. Overall conception rates between herds differed by less than 0.5 percentage points.

Averaged across all treatment protocols, conception rates among lactation numbers tended to differ. No treatment × lactation number interaction was detected. First-lactation cows tended (P = 0.10) to have greater conception rates than did second-lactation cows (34.5 vs. 27.7%), and cows in their third or greater lactation tended (P = 0.08) to differ from conception rates of second-lactation cows (34.7 vs. 27.7%). Our results tend to confirm those in a recent study of 1,584 lactating cows, in which first-lactation cows had greater conception rates than older cows when all inseminations were performed after an Ovsynch protocol. Further, less-fertile, older cows also produce more milk, which may account for some reduction in conception rates, although increased milk yields are confounded with age. That second-lactation cows had poorer conception rates in our study indicates that greater attention must be addressed to factors known to influence subsequent fertility, such as body condition and feed intake, and to other health issues during first lactations, first dry and subsequent transition periods, and early postpartum after second calvings.

We have demonstrated that one of the challenges in implementing various reproductive-management schemes is compliance to protocols. Nearly 19% of the cows were inseminated off-protocol. This is not an issue of concern for those cows in which inseminations occurred after detected estrus, but when TAI was administered to cows that received an improper injection sequence, pregnancy outcomes were compromised. Most serious infractions included those in which injections were given out of sequence, were given on the wrong dates, or were not given as designed. Compliance is difficult to monitor because documentation may be inaccurate or missing.

Our results clearly show that cows inseminated after detected estrus at similar DIM are more fertile than those receiving TAI. Nonetheless, given the poor rates of detected estrus (including missed observations and lack of estrus expression), TAI has proved to be an important tool for achieving pregnancies. In many studies, conception rates are nearly equal to, or greater than, for cows inseminated after estrus, but pregnancy rates (no. of pregnancies ÷ no. of cows attempted to AI) are often similar or greater because proportionally more cows of similar fertility are inseminated.

For cows inseminated at estrus at various DIM, those inseminated after 75 DIM had greater conception rates than those inseminated before 60 DIM. Establishing the appropriate VWP for individual herds is essential. Herd history for pregnancy outcomes after first services can be examined to determine if there is justification for delaying inseminations to achieve improved conception rates. In earlier studies in which no ovulation control was employed, various VWP were tested. Most studies demonstrated nearly similar conception rates for cows inseminated earlier versus later postpartum, but cows inseminated earlier generally required more services per pregnancy, partly because that measure (services per pregnancy) does not account for services made for cows that fail to conceive and are eventually culled.

Evaluation of synchrony protocols should include reproductive performance traits (e.g., herd estrus-detection rates) in addition to costs of administering protocols. A recent study found that Ovsynch used to initiate first services improved reproductive performance in two herds (reduced days to first services and days open; reduced culling for infertility in one herd), but AI based on detected estrus was economically superior in another herd, whereas Ovsynch was superior in the second herd because of poorer estrus-detection rates. Days open and culling were the major cost factors of those evaluated in their economic analysis. Inseminations associated with Ovsynch (TAI), compared with those made after detected estrus in response to $PGF_{2\alpha}$, have greater impact on net returns in summer months than during cooler months when estrus-detection rates tend to be greater.

Declining conception rates have been reported for lactating dairy cows since the 1950s, in the face of milk yields per cow that have increased 3.3 times. Our results indicate that the VWP should be extended in some herds to allow for improved fertility that may occur by delaying inseminations. Because of ovulation control and the benefits of increased persistency of lactation for cows treated with bST, a shorter VWP seems less critical, particularly when a longer VWP may result in improved pregnancy outcomes. Using ovulation control prevents prolonged and excessively variable intervals to first services. Further, and more important, because fewer than half of cows conceive at first AI, use of various tested resynchronization protocols for cows diagnosed not pregnant guarantees that cows are re-inseminated within 2 to10 days of their not-pregnant diagnosis.

Table 1. Conception Rates at First Services in Lactating Dairy Cows After Various Protocols

	Protocol[1]						
			2×PGF +	Heatsynch (HS)		Ovsynch (OVS)	
Item	1×PGF	2×PGF	GnRH ± PGF	Estrus	TAI	Estrus	TAI
No. of cows	145	727	96	212	154	43	271
Average DIM at AI	54 ± 0.4 ^{a} (40-89)	59 ± 0.2 ^{b} (51-102)	74 ± 0.5 ^{c} (65-95)	76 ± 0.3 ^{d} (71-118)	76 ± 0.4 ^{d} (73-106)	77 ± 0.7 ^{d} (73-84)	77 ± 0.3 ^{d} (73-110)
Pregnancy rate[2], %	22.6	33.0	32.1	44.6	21.1	48.7	24.4
Orthogonal contrasts:							
1×PGF vs. 2×PGF	22.6* (145)	33.0 (727)					
2×PGF vs. 2×PGF + GnRH ± PGF		33.0 (727)	32.1 (96)				
1×PGF + 2×PGF + 2×PGF + GnRH ± PGF vs. HS + OVS		28.3 (968)				31.5 (680)	
TAI (HS + OVS) vs. estrus (HS + OVS)				23.2*** (425)		45.3 (255)	
Heatsynch vs. Ovsynch				34.7 (366)		27.7 (314)	
Estrus only: 1×PGF + 2×PGF vs. HS + OVS		31.3** (872)				45.3 (255)	

[a,b,c,d] Means having different superscript letters differ (P<0.01).
*P<0.05.
**P<0.01.
***P<0.001.
[1]Cows were inseminated after a single injection of $PGF_{2\alpha}$ (1×PGF); after the second of two injections of $PGF_{2\alpha}$ given 14 days apart (2×PGF); after the first GnRH injection of Ovsynch or Heatsynch (some received PGF 7 days later and were inseminated that day; 2×PGF + GnRH ± PGF); after estrus induced by estradiol cypionate (ECP) injection of Heatsynch (GnRH injection 7 d before $PGF_{2\alpha}$ and ECP given 24 hours after $PGF_{2\alpha}$); timed AI at 48 hours after the $PGF_{2\alpha}$ injection of Heatsynch; after estrus following the $PGF_{2\alpha}$ injection of Ovsynch (injection of GnRH 7 days before and 48 hours after $PGF_{2\alpha}$); and timed AI at 12 to 18 hours after the second GnRH injection of the Ovsynch protocol.
[2]Means are based on analyses by logistic regression.