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Effect of timing of Cosynch on fertility of lactating Holstein cows after first postpartum and Resynch timed-AI services

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Abstract

To compare two intervals from the $PGF_{2\alpha}$ injection to the second GnRH injection + timed artificial insemination (TAI) of Ovsynch, lactating Holstein cows received their first postpartum TAI after Presynch + Ovsynch ($n = 352$) and second and greater postpartum TAI after resynchronization of ovulation using Ovsynch (Resynch; $n = 458$). Each week, cows housed in each of four breeding pens were randomized by breeding pen to receive the second GnRH injection of Presynch + Ovsynch or Resynch and TAI either 48 h (Cosynch 48; $n = 382$) or 72 h (Cosynch 72; $n = 428$) after the $PGF_{2\alpha}$ injection of Ovsynch or Resynch. Overall, pregnancies per AI (P/AI) did not differ for cows receiving Cosynch 48 (29%) versus Cosynch 72 (33%). Furthermore, treatment did not affect P/AI for cows receiving first postpartum TAI after Presynch + Ovsynch, for cows receiving second and greater TAI after Resynch, or the proportion of female calves born. In conclusion, delaying the second GnRH injection and TAI from 48 to 72 h after the $PGF_{2\alpha}$ injection of Ovsynch did not affect P/AI or calf sex ratio. The lack of a difference in fertility between these Cosynch protocols may offer more flexibility for implementing a systematic synchronization protocol when a Cosynch strategy is used.

Keywords: Dairy cow; Ovsynch; Cosynch; Fertility; Timed AI

1. Introduction

Successful systematic synchronization programs for managing dairy cattle reproduction comprise three steps: (1) submission of all cows for first postpartum timed artificial insemination (TAI) service at the end of the voluntary waiting period, (2) identification of non-pregnant cows early after TAI and (3) rapid resubmission of cows failing to conceive to TAI to a second or greater AI service [1]. Adoption of hormonal synchronization programs, such as Ovsynch (a synchronization regimen using sequential injections of GnRH and $PGF_{2\alpha}$ to precisely time ovulation for TAI [2]) have become a valuable tool to dairy producers to improve AI submission rates. Use of Presynch (postpartum regimen using two injections of $PGF_{2\alpha}$ to synchronize estrous cycles before applying Ovsynch [3,4]) + Ovsynch effectively synchronizes ovulation for first postpartum TAI to accomplish Step 1 above, and Presynch + Ovsynch has been widely adopted by the dairy industry based on a survey in which 75% of dairy producers reported use of synchronization for submitting cows for first postpartum TAI [5]. For maximum efficiency, Steps 2 and 3 above can be combined by coupling a non-pregnant diagnosis with the first GnRH injection of Ovsynch, or administering GnRH to all cows 7 days before pregnancy diagnosis and coupling a non-pregnant diagnosis with $PGF_{2\alpha}$ administration (Resynch).

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Performing TAI 32 h after the second GnRH injection of Ovsynch resulted in reduced fertility compared with TAI performed from 0 to 24 h after the second GnRH injection of Ovsynch; however, there was a quadratic effect for greater fertility when TAI was performed 16 h after the second GnRH injection of Ovsynch [6]. Although the 16 h interval from the second GnRH injection to TAI yielded the greatest fertility, it is often difficult to implement this scheme under field conditions, especially on farms with a thrice-daily milking schedule. Systematic synchronization protocols must also be easily implemented within the daily tasks of the farm or the program will not succeed due to compliance failures [1]. Thus, many dairy producers opt to perform TAI at the same time as the second GnRH injection of Ovsynch, a strategy known as Cosynch because it eliminates one cow handling period and facilitates once-daily restraint of cows for administration of hormone injections and TAI. A modification of Cosynch, using a 72 h versus a 48 h interval from the $PGF_{2\alpha}$ injection to the second GnRH injection of Ovsynch + TAI improved fertility of cows submitted for first postpartum TAI using Presynch + Ovsynch [7]. In that study, however, all cows were submitted to Ovsynch and TAI after a Presynch protocol, and the authors speculated that the increase in fertility due to delaying the second GnRH injection and TAI may not occur for resynchronized cows [7].

The timing of AI in relation to the second GnRH injection of Ovsynch or detected estrus not only affects fertility, but may also alter the expected sex ratio of offspring. Cows conceiving to TAI administered concurrently with the second GnRH injection of Ovsynch or 32 h after the second GnRH injection of Ovsynch had a calf sex ratio that was skewed toward female calves (60% female) compared to the expected female sex ratio of 45.8% [6]. In addition, when nulliparous Holstein heifers received AI to detected estrus or after a modified Cosynch protocol, heifers conceiving to the Cosynch protocol had a greater proportion female fetuses diagnosed using ultrasound (58%) compared to heifers inseminated after a detected estrus (50%; [8]). Further data are needed to either support or refute these observations.

The primary objective of this study was to evaluate pregnancies per AI (P/AI) after synchronization of ovulation using one of two modifications of Ovsynch: (1) GnRH + TAI 48 h after $PGF_{2\alpha}$ (Cosynch 48) or (2) GnRH + TAI 72 h after $PGF_{2\alpha}$ (Cosynch 72). A secondary objective was to evaluate P/AI to Cosynch 48 and Cosynch 72 based on synchronization protocol (Presynch + Ovsynch versus Resynch). Finally, the proportion of female calves resulting from Cosynch 48 to Cosynch 72 TAI was compared. Our hypothesis was that fertility would be greater for Cosynch 72 after Presynch + Ovsynch but not for Resynch TAI, and that a greater proportion of female calves would result from Cosynch 48 than Cosynch 72 TAI.

2. Materials and methods

Lactating Holstein dairy cows on a commercial dairy farm comprising approximately 3000 lactating cows located in west-central Michigan were enrolled into this study from February to April, 2004. Cows were housed in free-stall barns and were fed a total mixed ration with ad libitum access to feed and water. Lists for scheduled injections and pregnancy examinations for individual cows were generated weekly using a commercial on-farm computer software program (Dairy Comp 305, Valley Agricultural Software, Tulare, CA, USA). This program also was used to track and record treatment groups, reproductive outcomes, individual cow events and monthly milk production records for each cow enrolled in the experiment. Cows assigned to the study were coded by treatment at AI. Data from ‘‘cowfile’’ archives were transferred into a computer spreadsheet program (Microsoft Excel 2002, Microsoft Corporation, Redmond, WA, USA) for organization and manipulation of data before statistical analysis using SAS [9].

2.1. Experimental design

Cows enrolled into this experiment were housed in freestall barns in each of four breeding pens (n = 25, 20, 35 and 22 TAI/pen/week). All cows were submitted for TAI after receiving a hormonal ovulation synchronization protocol without detection of estrus. Approximately half (n = 382) of the cows received Cosynch 48 (GnRH + TAI 48 h after $PGF_{2\alpha}$), whereas the remainder (n = 428) of cows received Cosynch 72 (GnRH + TAI 72 h after $PGF_{2\alpha}$).

Cows receiving their first postpartum AI (n = 352) were synchronized using Presynch + Ovsynch as follows: 25 mg of $PGF_{2\alpha}$ (38 $\pm$ 3 and 52 $\pm$ 3 days; 5 mL of Lutalyse; Pfizer Animal Health, New York, NY, USA), 100 μg GnRH (66 $\pm$ 3 days; 2 mL of Cystorelin; Merial, Ltd., Duluth, GA, USA), 25 mg of $PGF_{2\alpha}$ (73 $\pm$ 3 days) and 100 μg of GnRH (75 $\pm$ 3 days, Cosynch 48; 76 $\pm$ 3 days, Cosynch 72) postpartum, with TAI immediately after the second GnRH injection of Ovsynch (i.e., Cosynch).

Second and greater TAI were conducted using Resynch as follows: 100 μg GnRH (66 ± 3 days), 25 mg of $PGF_{2\alpha}$ (73 ± 3 days) and 100 μg of GnRH (75 ± 3 days, Cosynch 48; 76 ± 3 days, Cosynch 72) postpartum, with TAI immediately after the second GnRH injection of Ovsynch (i.e., Cosynch). All previously inseminated cows received 100 μg GnRH 32 days (if previous TAI was Cosynch 72) or 33 days (if previous TAI was Cosynch 48) after AI to initiate Resynch regardless of their pregnancy status, which was unknown at the time of GnRH administration. Pregnancy status was assessed 39–40 days after TAI by transrectal palpation, which was conducted by the herd veterinarian, and cows diagnosed non-pregnant ($n = 458$) received $PGF_{2\alpha}$ to continue the Resynch protocol.

Each week, cows within a weekly breeding group were assigned by breeding pen so that two of the breeding pens received the Cosynch 48 treatment and two of the breeding pens received the Cosynch 72 treatment. In this way, cows were managed as groups to receive the first GnRH injection and the $PGF_{2\alpha}$ injection of Ovsynch and Resynch on Tuesdays, and GnRH + TAI on Thursdays (Cosynch 48) or Fridays (Cosynch 72). Treatments were alternated each week among the four breeding pens so that previously inseminated cows receiving second or greater TAI services did not repeat the same treatment consecutively. A total of eight weekly replicates were conducted to complete the experiment.

2.2. Statistical analyses

Dichotomous data were analyzed using PROC LOGISTIC of SAS [9]. A multivariate logistical regression model was developed to analyze the effects of the categorical variables treatment (Cosynch 48 versus Cosynch 72), parity (primiparous versus multiparous), AI technician, breeding pen, experimental week and synchronization protocol (Presynch + Ovsynch versus Resynch). Days in milk (DIM) at TAI was also included as a continuous variable except in the model for all TAI's because DIM was confounded with synchronization protocol. In addition, the two-way interactions of experimental week by breeding pen, treatment by protocol and treatment by parity were included in the statistical models. Separate multivariate logistic regression models were developed to test the treatment effect for Presynch + Ovsynch TAI services, Resynch TAI services and all TAI services. The effect of treatment on calf sex ratio was also evaluated by using the categorical variables parity, experimental week, AI technician, breeding pen, synchronization protocol and the two-way interactions of experimental week by breeding pen and treatment by synchronization protocol.

All multivariate logistical regression models were constructed using a backward selection procedure with treatment retained as a fixed factor in each of the models [10]. A Wald statistic criterion of $P < 0.15$ was set for including variables in the model. Data are presented as percentages and proportions with P-values for main effects and interactions derived from the multivariate logistical regression analysis.

3. Results

3.1. Effect of treatment on fertility to Presynch + Ovsynch

For Presynch + Ovsynch TAI services, variables remaining in the logistical regression model after the backwards selection procedure included treatment, experimental week and DIM. Although treatment and days in milk were retained in the statistical model after the backwards selection procedure, neither effect was significant ($P > 0.13$), and only week of TAI affected ($P = 0.01$) pregnancy outcomes. For cows receiving first postpartum TAI after Presynch + Ovsynch, P/AI did not differ ($P = 0.13$) between treatments, and was 29.5% (43/146) for Cosynch 48 and 36.9% (76/206) for Cosynch 72 (Table 1).

3.2. Effect of treatment on fertility to Resynch

For the comparison of Cosynch 48 and Cosynch 72 treatments for Resynch TAI services, variables remaining in the logistical regression model after the backwards selection procedure included treatment, parity, experimental week and DIM. When cows received Resynch for second and greater postpartum TAI, parity tended ($P = 0.09$) to affect P/AI, with greater P/AI for primiparous (35.9%; 42/117), than for multiparous (26.4%; 90/341) cows (Table 1); however, the treatment by parity interaction was not significant ($P > 0.90$). There was no difference ($P = 0.93$) in P/AI between Cosynch 48 (28.0%; 66/236) and Cosynch 72 (29.7%; 66/222) treatments for cows receiving TAI after Resynch, but there tended to be an effect of week of TAI ($P = 0.06$) and DIM ($P < 0.06$) on fertility.

3.3. Overall effect of treatment on fertility

For the analysis of the effect of treatment on P/AI for all TAI services, variables retained in the logistical

Table 1
Effect of timing of the second GnRH injection + timed artificial insemination (TAI) of Ovsynch on pregnancies per AI (P/AI) for lactating Holstein cows

Item	Treatment				*P*-value		
	Cosynch 48[a]		Cosynch 72[b]		Treatment	Parity	Treatment × Parity
	Primiparous	Multiparous	Primiparous	Multiparous			
Presynch[c]	34.1 (15/44)	27.5 (28/102)	40.6 (39/96)	33.6 (37/110)	0.13	0.91	0.95
Resynch[d]	39.6 (19/48)	25.0 (47/188)	33.3 (23/69)	28.1 (43/153)	0.93	0.09	0.94
Overall	37.0 (34/92)	25.9 (75/290)	37.6 (62/165)	30.4 (80/263)	0.30	0.01	0.71

[a] Cows received GnRH, Day 0; $PGF_{2\alpha}$, Day 7; GnRH + TAI 48 h after $PGF_{2\alpha}$.
[b] Cows received GnRH, Day 0; $PGF_{2\alpha}$, Day 7; GnRH + TAI 72 h after $PGF_{2\alpha}$.
[c] Cows received their first postpartum timed AI at 75 ± 3 DIM after Presynch ($PGF_{2\alpha}$ 38 ± 3 and 52 ± 3 DIM) followed by Ovsynch (GnRH, Day 0; $PGF_{2\alpha}$, Day 7; GnRH + TAI, Days 9–10) initiated 14 days after the second Presynch injection.
[d] All previously inseminated cows received GnRH 39–40 days after TAI to resynchronize ovulation. Cows were diagnosed non-pregnant by transrectal palpation 46–47 days after TAI, and only non-pregnant cows continued the Ovsynch protocol ($PGF_{2\alpha}$, at non-pregnant diagnosis; GnRH + TAI 2–3 days later).

regression model after the backwards selection procedure were week of TAI and parity. When all TAI services were combined, Cosynch 48 resulted in similar (*P* = 0.30) fertility, 28.5% (109/382), compared to Cosynch 72 at 33.2% (142/428; Table 1). Only parity affected (*P* = 0.01) P/AI, with primiparous cows having 37.4% (96/257) P/AI compared to 28.0% (155/553) for multiparous cows.

3.4. Effect of treatment on calf sex ratio

Only treatment remained in the logistical regression model after the backwards stepwise selection procedure for offspring resulting from Presynch + Ovsynch or Resynch TAI. For the overall comparison of Cosynch 48 to Cosynch 72, treatment and synchronization protocol remained in the logistical regression model after the backwards selection procedure.

Percentages and proportions for the proportion of female calves resulting from Cosynch 48 to Cosynch 72 treatments are presented in Table 2. For cows receiving first postpartum TAI after Presynch + Ovsynch, the proportion of female calves born did not differ (*P* = 0.38) between treatments, and was 46.4% (13/28) for Cosynch 48 and 57.1% (24/42) for Cosynch 72. For Resynch TAI services there was no difference (*P* = 0.97) in the proportion of female calves born to Cosynch 48, 40.0% (14/35), and Cosynch 72, 39.6% (19/48). When all TAI were combined, there was no difference (*P* = 0.57) between Cosynch 48 (42.9%; 27/63) and Cosynch 72 (47.8%; 43/90) treatments in the proportion of female calves born.

4. Discussion

4.1. Effect of treatment on fertility to Presynch + Ovsynch

For cows receiving first postpartum TAI after Presynch + Ovsynch, P/AI did not differ between treatments. This result from the present study does not agree with that reported by Portaluppi and Stevenson [7] in which cows synchronized using Presynch + Ovsynch and receiving the second GnRH + TAI at 72 h after the $PGF_{2\alpha}$ injection of Ovsynch resulted in greater fertility

Table 2
Effect of timing of the second GnRH injection + TAI of Ovsynch on the proportion of female calves in lactating Holstein cows

Item	Treatment		*P*-value
	Cosynch 48[a]	Cosynch 72[b]	
Presynch[c]	46.4 (13/28)	57.1 (24/42)	0.38
Resynch[d]	40.0 (14/35)	39.6 (19/48)	0.97
Overall	42.9 (27/63)	47.8 (43/90)	0.57

[a] Cows received GnRH, Day 0; $PGF_{2\alpha}$, Day 7; GnRH + timed AI 48 h after $PGF_{2\alpha}$.
[b] Cows received GnRH, Day 0; $PGF_{2\alpha}$, Day 7; GnRH + timed AI 72 h after $PGF_{2\alpha}$.
[c] Cows received their first postpartum timed AI at 75 ± 3 DIM after Presynch ($PGF_{2\alpha}$ 38 ± 3 and 52 ± 3 DIM) followed by Ovsynch (GnRH, Day 0; $PGF_{2\alpha}$, Day 7; GnRH + timed AI, Days 9–10) initiated 14 days after the second Presynch injection.
[d] All previously inseminated cows received GnRH 39–40 days after TAI to resynchronize ovulation. Cows were diagnosed non-pregnant by rectal palpation 46–47 days after TAI, and only non-pregnant cows continued the Ovsynch protocol ($PGF_{2\alpha}$, at non-pregnant diagnosis; GnRH + timed AI 2–3 days later).

than GnRH + TAI 48 h after $PGF_{2\alpha}$ combined with GnRH 48 h + TAI 16 h later. Furthermore, fertility to Cosynch 72 after Presynch + Ovsynch was less compared to cows receiving the second GnRH injection of Ovsynch 56 h after $PGF_{2\alpha}$ and TAI 16 h later, and tended to be less than Cosynch 48 [11]. Thus, the greatest fertility among TAI protocols evaluated thus far is achieved when cows are treated with GnRH 16 h before TAI in comparison to Cosynch protocols, regardless of the Cosynch protocol used.

Our initial hypothesis was that cows treated with Presynch + Ovsynch would be less likely to initiate estrus within 2 days after $PGF_{2\alpha}$ administration because a greater proportion of cows would be expected to ovulate in response to the first GnRH injection of Ovsynch, making both a 48 and 72 h interval from the $PGF_{2\alpha}$ injection to the second GnRH injection + TAI of Ovsynch feasible. In a previous study, none of the cows receiving Presynch + Ovsynch were observed in estrus within 48 h after treatment with $PGF_{2\alpha}$, whereas over 60% were detected in estrus 3 days after $PGF_{2\alpha}$ treatment [12]. The Cosynch 72 treatment may result in greater P/AI to Presynch + -Ovsynch than the Cosynch 48 treatment due to cows having an endogenous LH surge but failing to ovulate before the GnRH injection and TAI, thereby resulting in a more optimal timing of AI in relation to ovulation [6].

4.2. Effect of treatment on fertility to Resynch

Initiating Resynch 32–33 days after TAI should synchronize cows to receive the first GnRH injection of Ovsynch on or before day 12 of the estrous cycle, assuming a 21–23 days estrous cycle length. Although this interval would appear to initiate Ovsynch at a similar stage of the estrous cycle as cows receiving Presynch + Ovsynch, 16–22% of cows lack a CL 33 days after TAI [1,13], suggesting that there is significant biological variation and thus a lack of synchrony among groups of synchronized cows 33 days after an initial TAI. Based on preliminary data, Cosynch 72 resulted in reduced fertility compared with Cosynch 48 for cows that were not presynchronized before initiating Ovsynch [7]. Because there appears to be more variation in the stage of the estrous cycle when initiating Resynch versus Presynch + Ovsynch, one of our concerns was that cows receiving Cosynch 72 may express estrus and ovulate before administration of the second GnRH injection and TAI. Cows receiving TAI after ovulation has occurred produce fewer pregnancies and have greater pregnancy loss compared to cows that receive TAI before ovulation has occurred [6].

Despite our concerns, fertility was similar ($P = 0.93$) for Resynch cows treated with Cosynch 48 versus Cosynch 72. In agreement with these results, Brusveen et al. [11] reported that fertility did not differ between cows receiving Cosynch 48 and Cosynch 72 in which Resynch was initiated 31–33 days after TAI. By contrast, administration of the second GnRH injection of Ovsynch 56 h after $PGF_{2\alpha}$ and TAI 16 h later resulted in significantly greater fertility than either of the Cosynch treatments [11].

4.3. Effect of treatment on calf sex ratio

Cows conceiving to TAI administered concurrently with the second GnRH injection of Ovsynch or 32 h after the second GnRH injection of Ovsynch had a calf sex ratio that was skewed toward female calves (60% female) compared to the expected female sex ratio of 45.8% [6]. In addition, when nulliparous Holstein heifers received AI to detected estrus or after a modified Cosynch protocol, heifers conceiving to the Cosynch protocol had a greater proportion female fetuses diagnosed using ultrasound (58%) compared to heifers inseminated after a detected estrus (50%; [8]). When bovine cumulus–oocyte complexes were aspirated and placed in fertilization medium at the time of in vitro fertilization (IVF) or 8 h later, a greater proportion of female embryos resulted from the early IVF treatment (62%) compared to the later IVF treatment (38%; [14]). Although the second GnRH injection and TAI are administered concurrently for both the Cosynch 48 and Cosynch 72 treatments, there is a greater risk for cows receiving Cosynch 72 to have an endogenous LH surge before treatment with GnRH. Therefore, we hypothesized that Cosynch 48 could result in a greater proportion of female offspring than Cosynch 72. Nonetheless, no difference in calf sex ratio was detected in the present study. Interestingly, there was a tendency for synchronization protocol (Presynch + Ovsynch versus Resynch) to affect the proportion of female offspring, with more female calves resulting after Presynch + -Ovsynch than Resynch TAI. This observation was unexpected, but may still support the hypothesis that AI early in relation to ovulation results in a greater proportion of female calves. Presynch is intended to result in fewer cows in estrus before GnRH + TAI thereby increasing the likelihood of TAI occurring early in relation to ovulation.

4.4. Conclusions and implications

Contrary to our hypothesis, delaying the second GnRH injection + TAI to 72 h after $PGF_{2\alpha}$ following Presynch + Ovsynch failed to improve fertility to TAI. Likewise, P/AI after Resynch and all TAI did not differ between Cosynch 48 and Cosynch 72 treatments. The sex ratio of offspring also did not differ based on timing of the second GnRH + TAI of Cosynch. Although modifying the timing of the second GnRH + TAI of Cosynch failed to improve P/AI, the lack of a difference in fertility between these protocols may offer more flexibility for implementing a systematic synchronization protocol when a Cosynch strategy is used.

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