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Effect of Pretreatment with Prostaglandin $F_{2\alpha}$ Before Resynchronization of Ovulation on Fertility of Lactating Dairy Cows

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ABSTRACT

Our objective was to assess the effect of pretreatment with $PGF_{2\alpha}$ 12 d before initiation of a protocol for resynchronization of ovulation (Resynch) using an Ovsynch protocol. Lactating Holstein cows diagnosed not pregnant 31 d after a timed artificial insemination (TAI) were randomly assigned to initiate the Resynch protocol 32 d after TAI (n = 255; RES), or receive 25 mg of $PGF_{2\alpha}$ 34 d after TAI and initiate the Resynch protocol 12 d later at 46 d after TAI (n = 272; PGF+RES). Within each treatment, a subset of cows were examined using transrectal ultrasonography to determine ovulatory response to the first GnRH injection of the Resynch protocols or a blood sample was collected to determine serum progesterone (P_4) at initiation of the Resynch protocol, or both. Overall, PGF+RES cows had more pregnancies per artificial insemination (P/AI) than RES cows 66 d after TAI (35.2 vs. 25.6%), whereas pregnancy loss from 31 to 66 d after TAI was greater for RES than PGF+RES cows (17.1 vs. 7.6%). Although P/AI was greater for cows with high (≥1.0 ng/mL) vs. low (<1.0 ng/mL) P_4 at the first GnRH injection of the Resynch protocols, treatment did not affect the proportion of cows with low P_4 at the first GnRH injection of the Resynch protocols. Overall, no effect of treatment on ovulatory response to the first GnRH injection of the Resynch protocols was detected. We conclude that pretreatment with $PGF_{2\alpha}$ 12 d before initiation of the Resynch protocol increased P/AI 66 d after TAI for cows with serum P_4 concentration >1.0 ng/mL at the first GnRH injection of the Resynch protocol and decreased pregnancy loss from 31 to 66 d after TAI. This modified resynchronization protocol may be a useful strategy for reproductive management of lactating dairy cows.
Key words: dairy cow, Resynch, fertility, pregnancy loss

INTRODUCTION

Use of Presynch (Moreira et al., 2001) and Ovsynch (Pursley et al., 1997a) protocols and timed artificial insemination (**TAI**) have dramatically improved service rate to first AI and reduced the negative impact of poor expression and detection of estrus on dairies. Systematic strategies to resynchronize cows failing to conceive to first postpartum TAI are only now beginning to be developed and evaluated. Conception rate to first postpartum TAI is around 35% (Pursley et al., 1997a,b; Fricke et al., 2003), resulting in a large proportion of nonpregnant cows that need to be resynchronized after their first postpartum TAI. Many dairy producers have used Ovsynch for resynchronizing ovulation (i.e., Resynch) for cows diagnosed not pregnant to a previous TAI; however, the optimal timing of this strategy has not been well established.

One strategy used to increase conception rate to TAI after a Resynch protocol has been to choose intervals after TAI to initiate the Resynch protocol based on assumptions regarding the physiology of the estrous cycle (Fricke et al., 2003; Sterry et al., 2006a). Assuming an estrous cycle length of 21 to 24 d, initiation of a Resynch protocol 32 to 33 d after TAI should ensure that the first GnRH injection of the Resynch protocol is given between d 8 to 12 of the estrous cycle, a stage of the cycle when a corpus luteum (**CL**) should be present, resulting in greater conception rate when Ovsynch is initiated (Vasconcelos et al., 1999; Moreira et al., 2000). Despite this assumption, 16 to 22% of cows lacked a CL 33 d after TAI (Fricke et al., 2003; Sterry et al., 2006a) suggesting that there is significant variability in the stage of the estrous cycle, cyclicity status, or both, among cows 33 d after synchronization using Presynch + Ovsynch and TAI. Potential reasons for this variability among cows include normal variation among cows in length of the estrous cycle, the occurrence of pregnancy loss, lack of synchrony of ovarian function after the initial Presynch + Ovsynch protocol, or a combination of these.

Based on problems with the assumptions in the first approach, an alternative approach might be to presyn-

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chronize cows before initiation of the Resynch protocol. A common protocol to improve conception rate to Ovsynch at first postpartum TAI is to presynchronize groups of cows using 2 injections of $PGF_{2\alpha}$ administered 14 d apart with the second $PGF_{2\alpha}$ injection administered 12 to 14 d before the first GnRH injection of Ovsynch (Moreira et al., 2001; Navanukraw et al., 2004). After the second injection of $PGF_{2\alpha}$ of a Presynch + Ovsynch protocol, 90 to 95% of cyclic cows were detected in estrus within 7 d (Moreira et al., 2001). Thus, initiation of Ovsynch 12 d after treatment with $PGF_{2\alpha}$ would result in initiation of Ovsynch during the early to midluteal stages of the estrous cycle, thereby improving conception rate to TAI.

The objective of this study was to compare a commonly used resynchronization protocol initiated 32 d after TAI (**RES**) with a strategy in which cows were pretreated with a single injection of $PGF_{2\alpha}$ 12 d before initiation of the Resynch protocol (**PGF+RES**). Our hypothesis was that pretreatment with $PGF_{2\alpha}$ would increase the proportion of cows with a CL at initiation of the Resynch protocol and the likelihood that they would ovulate a follicle to the first GnRH injection of the Resynch protocol, thereby improving conception rate to resynchronization of ovulation and TAI.

MATERIALS AND METHODS

Farm Description and Data Collection

Lactating Holstein cows on a commercial dairy with 1,600 lactating cows located in south-central Wisconsin were enrolled into this study from September, 2005 to May, 2006. Cows were housed in free-stall barns and were milked thrice daily at approximately 8-h intervals. All cows received bST (Posilac, 500 mg, Monsanto Co., St. Louis, MO) beginning 57 to 70 d postpartum and continuing every 14 d throughout the study.

Cow lists for injection schedules, pregnancy examinations, and reproductive events for individual cows were generated, tracked, and recorded using a commercial on-farm computer software program (Dairy Comp 305, Valley Agricultural Software, Tulare, CA). Cows assigned to the study were identified and coded by treatment on the individual electronic card associated with each cow, and the cow file chronicling events for each cow was archived weekly to capture individual cow data throughout the study. Data from archived cow files were exported into a computer spreadsheet program (Excel 2002, Microsoft Corporation, Redmond, WA) for organization and manipulation of data before statistical analysis using SAS (2003).

Submission of Cows for First Postpartum TAI

Lactating Holstein cows (n = 843) with an average of 2.0 ± 1.2 lactations were enrolled into the study 1 wk before scheduled pregnancy diagnosis after receiving a hormonal synchronization protocol (Presynch + Ovsynch) using i.m. injections of 100 —g of GnRH (Cystorelin, Merial, Ltd., Duluth, GA) and 25 mg of $PGF_{2\alpha}$ (5 mL of Lutalyse, Pfizer Animal Health, New York, NY) as follows: $PGF_{2\alpha}$ (d 46 ± 3 and d 60 ± 3), GnRH (d 72 ± 3), $PGF_{2\alpha}$ (d 79 ± 3), GnRH 54 h after $PGF_{2\alpha}$ followed by TAI approximately 16 h later (d 82 ± 3 postpartum). A total of 7 of these cows were not submitted for pregnancy diagnosis because they either left the herd or missed their scheduled ultrasound examination.

Resynchronization Treatments

Pregnancy status was determined 31 d after Presynch + Ovsynch and TAI using a portable ultrasound scanner equipped with a 5 to 10 MHz linear-array transducer (Sonosite VET 180 plus, SonoSite Inc., Bothell, WA). The herd veterinarian, who had 8 yr experience using ultrasound for pregnancy diagnosis, conducted all pregnancy examinations 31 d after TAI throughout the experiment. Cows were blocked by parity (primiparous vs. multiparous) and were randomized and assigned to 1 of 2 resynchronization treatments for second and third postpartum TAI. Treatment codes for cows diagnosed pregnant were removed from the DC305 program so that they discontinued treatment, whereas cows diagnosed not pregnant continued the Resynch treatment.

Cows diagnosed not pregnant received Ovsynch for resynchronization of ovulation using i.m. injections of 100 μg of GnRH and 25 mg of $PGF_{2\alpha}$ and TAI as depicted in Figure 1. All cows received TAI approximately 16 h after the second GnRH injection of each resynchronization protocol. Cows enrolled in the first treatment (n = 255; RES) diagnosed not pregnant 31 d after TAI (Monday) received the first GnRH injection of the Resynch protocol 32 d after TAI (Tuesday), and this treatment was considered a control group. Cows enrolled in the second treatment (n = 272; PGF+RES) diagnosed not pregnant 31 d after TAI (Monday) received 25 mg of $PGF_{2\alpha}$ i.m. 34 d after TAI (Thursday) followed by the first GnRH injection of the Resynch protocol 12 d later at 46 d after TAI (Tuesday). In this way, all hormonal injections associated with synchronization and resynchronization protocols were restricted to 2 d of the week (Tuesdays and Thursdays) with pregnancy diagnoses occurring on Mondays and TAI occurring on Fridays. Cows failing to conceive to the first Resynch TAI remained in the same treatment group for a second Resynch TAI.

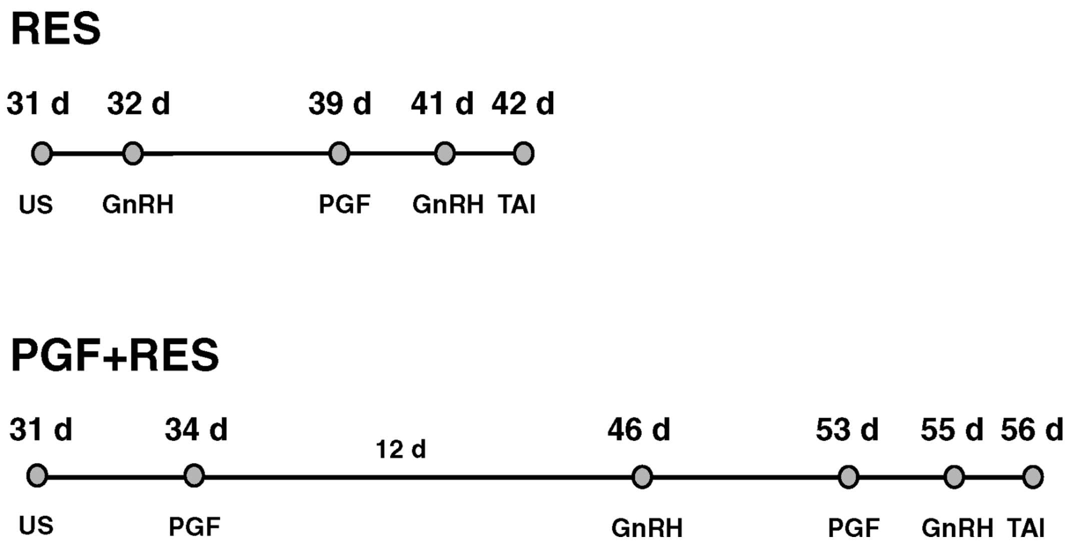

Figure 1. Schematic diagram of the timing and sequence of injections for the resynchronization of ovulation protocols. Cows diagnosed not pregnant 31 d after timed AI (TAI) using ultrasound (US) were assigned randomly to receive the first GnRH injection of the resynchronization protocol 32 d (RES) after a previous TAI or were pretreated with prostaglandin $F_{2\alpha}$ (PGF) 12 d before receiving the first GnRH injection of the resynchronization protocol (PGF+RES).

Pregnancies per AI (**P/AI**) was defined as the number of cows in each treatment diagnosed pregnant to TAI expressed as a percentage of cows within each treatment. Cows diagnosed pregnant 31 d after TAI were reexamined 66 d after TAI to determine pregnancy loss. Three cows left the herd before pregnancy reexamination (2 died and 1 was sold).

Blood Sampling and Progesterone Analysis

Blood samples for analysis of serum progesterone (**P_4**) concentration were collected via venipuncture of the median caudal vein or artery into evacuated tubes (Vacutainer, BD, Franklin Lakes, NJ) at the first GnRH injection of each Resynch treatment of a subset of cows in both treatments (251 RES and 253 PGF+RES cows). Blood samples were allowed to clot for 12 h at 4°C, centrifuged (1,935 × *g* for 15 min), and serum was harvested and stored at –20°C until assayed for P4 concentration using a solid-phase, no-extraction radioimmunoassay (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA). Intraassay and interassay coefficients of variation were 8.0 and 3.6%, respectively.

Ovarian Ultrasonography

Transrectal ultrasonography was conducted in a subgroup of cows (152 RES and 130 PGF+RES cows) using a portable scanner (Easi-scan, BCF Technology Ltd., Livingston, UK) fitted with a 7.5-MHz linear-array transducer to determine the ovarian structures at the first GnRH injection of the Resynch protocols and 7 d later at the $PGF_{2\alpha}$ injection. Diameter of the follicle and CL was estimated using on-screen background gridlines comprising squares with 10-mm sides as described previously (Rivera et al., 2006). Ovulation was defined as the presence of a follicle on the day of first ultrasound examination and presence of a CL in the same location of the ovary 7 d later at the second ultrasound examination. Because of missing data at the first GnRH injection or at $PGF_{2\alpha}$ injections, 25 cows were excluded from this analysis.

Statistical Analyses

Dichotomous data were analyzed using the Logistic procedure of SAS (2003). A multivariate logistical regression model was developed to analyze the effect of the categorical variables treatment (RES vs. PGF+RES), parity (primiparous vs. multiparous), resynch number (first vs. second), and all 2-way interactions of the explanatory variables with treatment on P/AI at 31 and 66 d after TAI and pregnancy loss.

The same multivariate logistical regression model was used for the analysis of the subgroup of cows that were examined with ultrasound at the first GnRH and $PGF_{2\alpha}$ injections of the Resynch protocols. The effect of treatment, Resynch number, and P_4 (<1.0 ng/mL vs. ≥1.0 ng/mL) on ovulatory response after the first GnRH injection of the Resynch protocols and the effect of treatment, ovulation after the first GnRH injection of the Resynch protocols, and presence of a CL at the $PGF_{2\alpha}$

injection of the Resynch protocols on P/AI 31 and 66 d after TAI were evaluated. Interaction of the explanatory variables with treatment was included in both statistical models. Because a total of 94 cows had missing blood samples thereby comprising a distinct subgroup of cows, a separate statistical model was developed to analyze the effect of treatment, low vs. high P_4 (<1.0 ng/mL vs. ≥1.0 ng/mL), and the interaction of treatment and P_4 class on P/AI 31 and 66 d after TAI and pregnancy loss.

All multivariate logistical regression models were constructed using a backward selection procedure with treatment retained as a fixed effect in each of the models. A Wald statistic criterion of $P < 0.15$ was set for including variables in the statistical model. Treatment differences with $P < 0.05$ were considered significant, and differences between $P > 0.05$ and $P < 0.15$ were considered statistical tendencies. Data are presented as percentages and proportions with *P*-values for main effects and interactions derived from the multivariate logistical regression analysis.

RESULTS AND DISCUSSION

Of the 843 cows enrolled into the study, 7 cows were not submitted for pregnancy diagnosis after Presynch + Ovsynch TAI because they either left the herd or missed their scheduled ultrasound examination. After exclusion of these 7 cows, P/AI 31 d after the Presynch + Ovsynch protocol was 37.0% (309/836) and was similar between primiparous 35.3% (120/340) and multiparous 38.1% (189/496) cows. By design, mean DIM at pregnancy diagnosis after the Presynch + Ovsynch protocol was similar ($P > 0.1$) between RES and PGF+RES cows (111.8 ± 0.3 vs. 112.0 ± 0.2, respectively).

After exclusion of cows diagnosed pregnant 31 d after first postpartum TAI (Table 1), a total of 527 nonpregnant cows remained in the study to receive RES (n = 255) and PGF+RES (n = 272) treatments. One hundred eleven cows enrolled in the study and diagnosed not pregnant at 31 d after first TAI did not complete the resynchronization protocol for the reasons described in Table 1. For the final analysis, a total of 198 cows were included in the RES treatment and 218 cows were included in the PGF+RES treatment of first resynchronization of ovulation. At the subsequent TAI, 43 and 35 cows were excluded from the RES and PGF+RES treatments, respectively resulting in 88 RES and 94 PGF+RES cows included in the final analysis for second resynchronization of ovulation (Table 1).

Implicit to the experimental design, RES cows had fewer ($P < 0.001$) DIM at second (RES = 122.8 ± 0.3, PGF+RES = 137.0 ± 0.2 d) and third (RES = 164.5 ± 0.3 and PGF+RES = 193.0 ± 0.5 d) postpartum AI than PGF+RES cows. Variables included in the logistical regression model and *P*-values for P/AI 31 and 66 d after TAI and pregnancy loss are summarized in Table 2. There was an effect of Resynch number on P/AI 31 d after TAI ($P < 0.05$) and a tendency for an effect of Resynch number 66 d after TAI ($P = 0.109$). Overall, there were more P/AI after first postpartum resynchronization of ovulation than second postpartum resynchronization of ovulation at 31 (37.5 vs. 29.1%) and 66 (32.6 vs. 26.0%) d after TAI. Because there was no interaction between treatment and Resynch number and because of the limited number of cows enrolled for a second postpartum resynchronization (RES = 88 and PGF + RES = 94), data from first and second resynchronization were combined for subsequent analyses.

Effect of Treatment on P/AI and Pregnancy Loss

At 31 d after TAI, there was a tendency ($P = 0.061$) for PGF+RES cows to have more P/AI than RES cows for first and second Resynch combined (38.5 vs. 31.1%, respectively; Table 3). In the present study, P/AI 31 d after TAI for PGF+RES cows was similar to (Fricke et al., 2003; Sterry et al., 2006a) or greater than (Bartolome et al., 2005a; Sterry et al., 2007) that reported in other studies when resynchronization of ovulation using Ovsynch was initiated approximately 33 d after TAI. At pregnancy diagnosis 66 d after TAI, PGF+RES cows had more ($P = 0.05$) P/AI than RES cows (35.2 vs. 25.6%, respectively; Table 3). Neither the effect of parity (primiparous vs. multiparous cows) nor the interaction of parity and treatment on P/AI 31 or 66 d after TAI were significant. This observation is inconsistent with studies in which parity and treatment by parity interactions have been reported to be significant (Sterry et al., 2006a, 2007). Specific reasons for this disparity are not known; however, pretreatment with $PGF_{2\alpha}$ before initiation of the resynchronization protocol may have minimized differences in follicular wave dynamics between primiparous and multiparous cows. Furthermore, the present study was conducted on a different dairy than the previous studies.

By contrast, pregnancy loss from 31 to 66 d after TAI was greater ($P < 0.05$) for RES cows than PGF+RES cows (17.0 vs. 7.6%, respectively; Table 3), and this difference was primarily due to differences in pregnancy loss after first resynchronization (19.7 vs. 6.8%, respectively; Table 3). This reduction in pregnancy loss in cows receiving the PGF pretreatment before the Resynch protocol was unexpected, and further studies are required to confirm this outcome and to discover the mechanisms that underlie this effect. One study reported greater pregnancy losses after a Resynch than a Presynch + Ovsynch protocol (Bartolome et al., 2005a);

Table 1. Outcomes for cows that were diagnosed not pregnant to a previous timed AI (TAI) but did not complete the Resynch protocols by treatment and Resynch number (first vs. second Resynch)[1]

	RES		PGF+RES	
Item	First Resynch	Second Resynch	First Resynch	Second Resynch
Cows pregnant to previous TAI, % (n/n)	38.1 (157/412)	33.8 (67/198)	35.8 (152/424)	40.8 (89/218)
Cows initially enrolled for Resynch (n)	255	131	272	129
Reasons for exclusion; n (%)				
Died	2 (0.8)	0 (0)	6 (2.2)	1 (0.8)
AI to detected estrus	11 (4.3)	16 (12.2)	22 (8.1)	17 (13.2)
Marked as "do not breed"	10 (3.9)	5 (3.8)	2 (0.7)	2 (1.6)
Incorrect injection sequence	5 (2.0)	0 (0)	9 (3.3)	1 (0.8)
Delayed Resynch start	18 (7.1)	10 (7.6)	1 (0.4)	3 (2.3)
Sold	4 (1.6)	4 (3.1)	7 (2.6)	9 (7.0)
Inconclusive pregnancy diagnosis	7 (2.7)	1 (0.8)	7 (2.6)	2 (1.6)
Inadvertently changed treatment[2]	—	7 (5.3)	—	0 (0)
Total cows excluded, n (%)	57 (22.4)	43 (32.8)	54 (19.9)	35 (27.1)
Total cows included in the analysis (n)	198	88	218	94

[1]RES cows received the first GnRH injection of the resynchronization protocol 32 d after TAI, whereas PGF+RES cows received a $PGF_{2\alpha}$ injection 34 d after TAI and received the first GnRH injection of the resynchronization protocol 46 d after TAI.

[2]Treatment code inadvertently removed from the on-farm computer management program during the course of the experiment.

however, other studies reported pregnancy losses of a lesser extent after resynchronization and closer to the losses after the PGF+RES treatment evaluated in the present study (Bartolome et al., 2005b; Sterry et al., 2006a). Nonetheless, differences in the experimental designs used among these studies preclude a direct comparison for reported pregnancy losses after resynchronization of ovulation.

One possible explanation for the reduced pregnancy loss for PGF+RES cows is that pretreatment with $PGF_{2\alpha}$ before the Resynch protocol may have had a direct beneficial effect on the uterine environment for cows undergoing early pregnancy loss after the preceding TAI. Pregnancy loss is more frequent before maternal recognition of pregnancy (d 16 to 19 of the estrous cycle; Santos et al., 2004). In addition, a positive effect of treatment with $PGF_{2\alpha}$ to synchronize estrous behavior on conception rate has been reported in cattle (Roche, 1976; Macmillan and Day, 1982).

Table 2. Variables included in the initial multivariate logistical regression model and *P*-values for pregnancies per artificial insemination (P/AI) 31 and 66 d after timed AI and pregnancy loss for lactating Holstein cows receiving first and second Resynch

	P/AI		
Variable	31 d	66 d	Pregnancy loss
Treatment	0.061*	0.012*	0.041*
Parity[1]	0.795	0.742	0.153
Resynch number[2]	0.049*	0.109*	0.572
Treatment × parity	0.410	0.588	0.821
Treatment × Resynch number	0.824	0.665	0.280

*Variables that remained in the final multivariate logistical regression model constructed using a backward selection procedure with treatment retained as a fixed factor in each of the models. A Wald statistic criterion of $P < 0.15$ was set for retaining variables in the final model.

[1]Primiparous vs. multiparous cows.

[2]First vs. second postpartum Resynch.

Taken together, the improvement in P/AI by pretreatment with $PGF_{2\alpha}$ has potential to improve reproductive performance on dairies that use a systematic synchronization and resynchronization approach for reproductive management. In addition, overall P/AI 31 d after TAI for PGF+RES cows (38.5%) was similar to that of cows submitted to first postpartum TAI after a Presynch + Ovsynch protocol (37.0%). Thus, the proportion of less fertile cows does not necessarily increase with each TAI attempt, but rather a reduction in synchrony of ovulation at second and greater TAI may account for most of this reduction in fertility. Thus, the similarity between conception rates at the first and subsequent TAI also suggests better resynchronization of ovulation with the PGF+RES treatment.

Role of Progesterone Concentration at Initiation of the Resynch Protocols

A key hypothesis of the present study was that pretreatment with $PGF_{2\alpha}$ 12 d before initiation of the Resynch protocol would increase the number of cows with high P_4 at initiation of the Resynch protocol. When resynchronization of ovulation was initiated 33 d after TAI, cows with a CL at the first GnRH injection tended to have more P/AI than cows lacking a CL (Fricke et al., 2003), and a similar effect was reported for cows receiving a Presynch + Ovsynch protocol (Sterry et al., 2006b). Cows with low P4 should be at either late or early stages of the estrous cycle or may represent a population of anovular cows. When an Ovsynch protocol

Table 3. Effect of treatment, parity (primiparous vs. multiparous), and Resynch number (first vs. second) on pregnancies per AI (P/AI) after timed AI (TAI) in lactating Holstein cows[1]

	RES		PGF+RES			*P*-value		
Item	Primiparous	Multiparous	Primiparous	Multiparous	Overall[2]	Treatment	Parity	Resynch
P/AI (%)								
31 d	28.7 (37/129)	33.1 (52/157)	39.6 (55/139)	37.6 (65/173)	31.1 vs. 38.5	0.061	0.795	0.049
66 d	25.0 (32/128)	26.1 (41/157)	37.0 (51/138)	33.7 (58/172)	25.6 vs. 35.2	0.012	0.742	0.109
Pregnancy loss (%)								
31 to 66 d	11.1 (4/36)	21.2 (11/52)	5.6 (3/54)	9.4 (6/64)	17.1 vs. 7.6	0.041	0.153	0.572

[1]RES cows received the first GnRH injection of the resynchronization protocol 32 d after TAI, whereas PGF+RES cows received a $PGF_{2\alpha}$ injection 34 d after TAI and received the first GnRH injection of the resynchronization protocol 46 d after TAI.

[2]Overall P/AI and pregnancy loss × treatment (RES vs. PGF+RES).

is initiated at these stages of the estrous cycle or in anovular cows, a reduction in conception rate to TAI has been reported (Vasconcelos et al., 1999; Gmen et al., 2003; Sterry et al., 2006b).

To evaluate the effect of serum P_4 at initiation of the Resynch protocol on P/AI, cows were classified by serum P_4 at the first GnRH injection of the Resynch protocols as either low (<1.0 ng/mL) or high (≥1.0 ng/mL) as described previously (Rivera et al., 2005). Overall, the proportion of cows with high P_4 at the first GnRH injection of the Resynch protocol did not differ ($P > 0.10$) between RES (n = 251, 76.1%) or PGF+RES (n = 253, 73.1%) cows. It is surprising that a similar percentage of cows in both treatments lacked a functional CL at initiation of the Resynch protocols especially because PGF+RES cows were treated with $PGF_{2\alpha}$ 12 d before the first GnRH injection. El-Zarkouny et al. (2004) reported that presynchronization with 2 injections of $PGF_{2\alpha}$ increased the proportion of cows with high P_4 (≥1.0 ng/mL) at the first GnRH injection of Ovsynch by 13 percentage points. By contrast, Moreira et al. (2001) did not observe an effect of a similar presynchronization protocol on the proportion of cyclic cows with high P_4 at the first GnRH injection of Ovsynch, although they did report that presynchronized cows had greater P_4 concentrations at the $PGF_{2\alpha}$ injection 7 d later. Nonetheless, the proportion of cows with low P_4 in the present study is similar to the 16 and 22% of cows reported to lack a CL at the first GnRH injection of a Resynch protocol (Fricke et al., 2003; Sterry et al., 2006a) and may support the observation that these cows were anovular at initiation of the Resynch protocols. Although results from these 2 methodologies (serum P_4 vs. ultrasound) are not perfectly correlated, the statistical agreement (kappa) between the presence or absence of a CL using transrectal ultrasonography and serum P_4 concentration at the ultrasound examination was 0.74, indicating a high level of agreement between these methods (Silva et al., 2007).

When the variable P_4 concentration at the first GnRH injection of the Resynch protocols was included in the statistical model to assess the effect of treatment and P_4 status on P/AI 31 and 66 d after TAI, cows with low P_4 at the first GnRH injection of the Resynch protocols had fewer P/AI at 31 ($P < 0.008$) and 66 ($P < 0.005$) d after TAI than cows with high P_4 regardless of resynchronization treatment (Table 4). Although this outcome was expected, the overall data on circulating progesterone from the present study did not provide a clear explanation for the improvement in P/AI observed for the PGF+RES treatment. Cows from the RES and PGF+RES treatments that had low P_4 at the first GnRH injection of the Resynch protocols (~25% of cows; Table 4) had low P/AI after the Resynch TAI, whereas the effect of pretreatment with $PGF_{2\alpha}$ on P/AI in the present study was only observed for cows with high P_4 at initiation of the Resynch protocols. An intriguing possibility to explain the lack of an effect of pretreatment with $PGF_{2\alpha}$ on the proportion of cows with low P_4 in the present study is that a proportion of cows are anovular at this time despite completing a synchronization protocol earlier postpartum; however, further research is needed to either confirm or refute this explanation.

Role of Ovulatory Response to the First GnRH Injection of the Resynch Protocols

In addition to P_4 status at initiation of the resynchronization protocols, ovulatory response to the first GnRH injection of the Resynch protocol could account for some of the effect of pretreatment with $PGF_{2\alpha}$ on P/AI, pregnancy loss, or both, in the present study. Because of missing data at the first GnRH or the $PGF_{2\alpha}$ injections, 25 cows were excluded from this analysis. Although no effect of treatment on ovulatory response to the first GnRH injection of the Resynch protocols was detected (Table 5), there was a treatment × P_4 status interaction on ovulatory response to the first GnRH injection when the analysis was restricted to cows in which the largest follicle was ≥10 mm in diameter, the minimum diameter threshold for acquisition of ovulatory capacity in dairy cattle (Sartori et al., 2001). Nonetheless, when all cows

Table 4. Effect of P_4 concentration (high vs. low)[1] at the first GnRH injection of the Resynch protocols on pregnancies per AI (P/AI) in lactating Holstein cows[2]

Item	RES		PGF+RES		Overall[3]	*P*-value		
	Low P_4	High P_4	Low P_4	High P_4		Treatment	P_4	Interaction
P/AI (%)								
31 d after TAI	25.0 (15/60)	34.0 (65/191)	25.0 (17/68)	41.6 (77/185)	25.0 vs. 37.8	0.178	0.008	0.483
66 d after TAI	20.0 (12/60)	27.9 (53/190)	20.6 (14/68)	38.6 (71/184)	20.3 vs. 33.2	0.045	0.005	0.363
Pregnancy loss (%)								
31 to 66 d	20.0 (3/15)	17.2 (11/64)	17.7 (3/17)	6.6 (5/76)	18.8 vs. 11.4	0.080	0.271	0.386

[1]Blood samples were collected at the first GnRH injection of each Resynch protocol and cows were classified as having low (<1.0 ng/mL) or high (≥1.0 ng/mL) P_4.

[2]RES cows received the first GnRH injection of the resynchronization protocol 32 d after TAI, whereas PGF+RES cows received a $PGF_{2\alpha}$ injection 34 d after TAI and received the first GnRH injection of the resynchronization protocol 46 d after TAI.

[3]Overall P/AI × P_4 concentration (low vs. high).

were included in the analysis regardless of follicular diameter there was a numerical difference in P/AI between cows in the PGF+RES treatment that did or did not ovulate to the first GnRH injection of the Resynch protocol, and the low number of observations in this analysis may have resulted in a type II statistical error.

When the effect of ovulatory response to the first GnRH injection of the Resynch protocols on P/AI 66 d after TAI was analyzed, ovulatory response did not affect P/AI 66 d after TAI, but there was a tendency for an interaction ($P = 0.068$) between treatment and ovulatory response to the first GnRH injection for cows in which the largest follicle was ≥10 mm (Table 6). Overall, PGF+RES cows that ovulated to the first GnRH injection of the Resynch protocols tended to have more P/AI than RES cows that ovulated and cows in both treatments that failed to ovulate to the first GnRH injection (Table 6).

In agreement with this observation, cows that ovulate after the first GnRH injection of Ovsynch had greater conception rates than cows that fail to ovulate to the first GnRH injection (Chebel et al., 2006). When Ovsynch was initiated at d 3 of the estrous cycle, there was a reduction in ovulatory response to the first GnRH injection of Ovsynch and a negative effect on embryo quality (Cerri et al., 2005). Thus, cows failing to ovulate to the first GnRH injection may fail to ovulate after the second GnRH injection of Ovsynch or may ovulate follicles that are either too small or too large, thereby reducing conception rate through compromised embryo quality. Beef cows that ovulate small follicles after TAI protocols had reduced conception rates (Perry et al., 2005), whereas ovulation of large follicles may compromise oocyte quality leading to a reduction in conception rate, impairment of embryo development, and increased pregnancy loss (Ahmad et al., 1995). Ovulatory follicle diameter may also be an indicator of the duration of follicular dominance, which can also affect fertility in cattle (Mihm et al., 1994). In the present study, the ovulatory response data may be confounded by the presence of anovular cows in both treatments. Indeed, the improvement in conception rate was only observed for cows with serum P_4 greater than 1.0 ng/mL at initiation of Resynch. Further studies will be required to determine the mechanism(s) that cause the improvement in P/AI to Resynch TAI after pretreatment with $PGF_{2\alpha}$.

Table 5. Effect of treatment and progesterone (P_4) concentration (high vs. low)[1] at the first GnRH injection of the Resynch protocols on ovulatory response of lactating Holstein cows for all cows and for cows in which the largest follicle was ≥10 mm[2]

Item	RES		PGF+RES		*P*-value		
	Low P_4	High P_4	Low P_4	High P_4	Treatment	P_4	Interaction
Ovulation (%)							
All cows	63.6 (21/33)	44.9 (48/107)	60.0 (18/30)	51.7 (45/87)	0.491	0.057	0.470
Follicle ≥10 mm	84.0 (21/25)	47.8 (44/92)	62.1 (18/29)	60.3 (44/73)	0.370	0.013	0.023

[1]Blood samples were collected at first GnRH injection of each Resynch protocol and cows were classified as having low (<1.0 ng/mL) or high (≥1.0 ng/mL) P_4.

[2]RES cows received the first GnRH injection of the resynchronization protocol 32 d after TAI, whereas PGF+RES cows received a $PGF_{2\alpha}$ injection 34 d after TAI and received the first GnRH injection of the resynchronization protocol 46 d after TAI.

Table 6. Effect of treatment and ovulatory response (yes vs. no) after the first GnRH injection of the Resynch protocols on pregnancies per AI (P/AI) 66 d after timed AI (TAI) in lactating Holstein cows for all cows and for cows in which the largest follicle was ≥10 mm[1]

	RES		PGF+RES		*P*-value		
Item	Yes	No	Yes	No	Treatment	Ovulation	Interaction
P/AI (%)							
All cows	27.8 (20/72)	31.4 (22/70)	44.6 (29/65)	30.9 (17/55)	0.090	0.945	0.232
Follicle ≥10 mm	27.9 (19/68)	32.7 (17/52)	45.3 (29/64)	26.2 (11/42)	0.449	0.290	0.068

[1]RES cows received the first GnRH injection of the resynchronization protocol 32 d after TAI, whereas PGF+RES cows received a $PGF_{2\alpha}$ injection 34 d after TAI and received the first GnRH injection of the resynchronization protocol 46 d after TAI.

CONCLUSIONS

Coupling a nonpregnancy diagnosis with a management decision to quickly reinseminate cows may improve reproductive efficiency by decreasing the interval between AI services. By contrast, early pregnancy loss and the effectiveness of resynchronization protocols initiated at different stages after a previous TAI may limit the effectiveness of resynchronization strategies. In the present study, PGF+RES cows had more P/AI 66 d after TAI compared with RES cows and less pregnancy loss from 31 to 66 d after TAI. It is unclear whether the improvement in P/AI observed for PGF+RES cows in the present study can compensate for the greater interval (14 d) between AI services. Based on pregnancy outcomes 66 d after TAI, pretreatment with $PGF_{2\alpha}$ 12 before initiation of the Resynch protocol yielded a 9.6 percentage point increase in P/AI compared with the control treatment or about 37.5% more pregnancies at each Resynch AI (9.6/25.6 = 37.5%). A comparison of the economic value of these protocols on dairy farms is complex and will require further research with a larger number of farms with differences in management practices, milk production, and lactation persistency in the economic models. Nonetheless, data from the present study support the concept that P/AI to TAI after resynchronization of ovulation can achieve a similar rate to that observed after a Presynch + Ovsynch protocol for first postpartum TAI. Future studies to confirm these results and investigate other methods for presynchronizing cows to improve P/AI to resynchronization of ovulation are warranted.

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