

# Resynchronization Strategies for Dairy Cows

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## Introduction

In contrast to grazing-based dairy systems, many confinement-based dairy systems in the U.S. have adopted systematic synchronization protocols and timed artificial insemination (TAI) for submitting cows for first postpartum AI service. Although reliance on synchronization of ovulation and TAI for improving service rate to first AI service reduces the impact of poor estrous detection, the improved AI submission rate to first TAI often is followed by a time lag exceeding 60 d before cows failing to conceive are detected and reinseminated. Because conception rates to TAI for dairy cows managed in confinement-based systems in the U.S. are reported to be 40 % or less (Pursley et al., 1997a,b; Fricke et al., 1998; Jobst et al., 2000), 60 % or more of the cows will fail to conceive and therefore require a resynchronization strategy for aggressively initiating subsequent AI services. Methods for early detection of nonpregnancy coupled with hormonal resynchronization systems that program nonpregnant cows to receive subsequent TAI services are now being developed and assessed so that systematic reproductive management programs can be implemented to aggressively manage reproduction (Fricke, 2002). A wide array of reproductive management strategies have been developed to fit the various dairy production systems that are found around the world, and the diversity among these strategies has been reviewed elsewhere (Lucy et al., 2004). Although studies have been conducted to resynchronize behavioral estrus among groups of previously inseminated cows (Chenault et al., 2003), the objective of this review is to overview strategies for resynchronization of ovulation that allow for TAI of cows failing to conceive to a prior AI service.

## Resynchronization of Ovulation (Resynch) using Ovsynch

Pursley et al. (1997a) were the first to compare a systematic reproductive management strategy without detection of estrus using Ovsynch for first and subsequent TAI services with standard reproductive management based on detection of estrus. Lactating dairy cows (n=333) from three commercial dairies were randomly assigned to receive standard reproductive management consisting of detection of estrus behavior, the AM/PM rule for timing of AI, and periodic use of PGF<sub>2α</sub> (Control) or Ovsynch and TAI without detection of estrus (Ovsynch). Cows assigned to the Ovsynch treatment initiated the first gonadotrophin releasing hormone (GnRH) injection of Ovsynch for resynchronization (i.e., Resynch) after a not-pregnant diagnosis using transrectal ultrasonography (US) 32 d after first postpartum TAI service. Cows remained in their respective treatments until diagnosed pregnant or culled from the herd. Although Ovsynch cows received each respective AI service earlier than Control cows, fertility for the first three AI services was similar between treatments (Table 1). This study demonstrated for the first time that reproductive performance could be improved using Ovsynch without reliance on visual detection of estrus compared to standard reproductive management using detection of estrus.

A subsequent Resynch trial that investigated use of Ovsynch for resynchronizing nonpregnant cows was a field trial in which cows of unknown pregnancy status that had been submitted for first postpartum TAI received GnRH 20 d after TAI followed by US and PGF<sub>2α</sub> administration to

Table 1. Median days postpartum (PP) at AI and pregnancies per AI (P/AI) in lactating Holstein cows inseminated after a detected estrus (Control) or synchronization of ovulation (Ovsynch) and timed AI (adapted from Pursley et al., 1997a).

Treatment	First AI		Second AI		Third AI	
	PP (d)	P/AI (%)	PP (d)	P/AI (%)	PP (d)	P/AI (%)
Control	83	39	128	45	170	61
Ovsynch	54	37	96	42	140	48
<i>P</i>	<0.001	>0.25	<0.001	>0.15	<0.001	>0.15

Table 2. Effect of resynchronization with GnRH 21 d after AI on pregnancies per AI (P/AI) and pregnancy loss (PL) in lactating dairy cows (adapted from Chebel et al., 2003).

Item	Control (n=295)	Resynch (n=290)	P
P/AI (%)			
28 d after AI	33.6	33.1	0.80
42 d after AI	26.8	27.0	0.98
PL (%)			
28 to 42 d after AI	17.9	17.9	0.73

nonpregnant cows 27 d after TAI (Moreira et al., 2000b). The advantage of this strategy was that Ovsynch could be initiated 7 d before a not-pregnant diagnosis by pre-treating cows with GnRH; thereby further reducing the interval between successive TAI services. Cows in the control group were reinseminated after a detected estrus. Blood samples were collected from cows 20 and 27 d after TAI to determine plasma progesterone ( $P_4$ ) concentration to assess pregnancy status. Cows not observed in estrus after an initial TAI, which had plasma  $P_4$  concentration  $> 2.0$  ng/ml were considered pregnant to the initial TAI; whereas cows not observed in estrus after TAI, which had plasma  $P_4$  concentration  $\leq 2.0$  ng/ml, were considered not pregnant to the initial TAI. Unexpectedly, a statistical interaction was detected for cows pre-treated with GnRH 20 d after TAI in which the estimated embryonic loss from 20 to 27 d after TAI based on plasma  $P_4$  concentrations was greater ( $P < 0.08$ ) for bST-treated pregnant cows pre-treated with GnRH (55.6 and 52.4 %) but not for non-bST-treated pregnant cows (39.8 and 44.8 %). Based on this observation, pre-treatment with GnRH before pregnancy diagnosis was discontinued as a Resynch strategy during the course of the trial (Moreira et al., 2000b).

Although the negative effect of GnRH on early pregnancy losses reported by Moreira et al. (2000b) was defined by an interaction with bST and by using an indirect assessment of pregnancy status based on plasma  $P_4$  concentration 20 d after TAI, these results caused many in the dairy industry to question the strategy of pre-treating cows of unknown pregnancy status with GnRH 7 d before pregnancy diagnosis using US in conjunction with a Resynch strategy. A follow-up study was conducted to assess the effect of GnRH pretreatment on fertility and pregnancy loss in lactating Holstein cows (Chebel et al., 2003). At 21 d after a pre-enrollment AI, cows (n=585) on two commercial dairies were randomized to receive either 100  $\mu$ g GnRH (Resynch) or no treatment (Control). Cows in the Resynch treatment diagnosed not-pregnant using US 28 d after the pre-enrollment AI received  $PGF_{2\alpha}$  and continued the Ovsynch protocol, whereas cows in the Control treatment initiated Ovsynch at a not-pregnant diagnosis using US 28 d

after the pre-enrollment AI. Using this experimental design, exposure of pregnant cows to GnRH pre-treatment was restricted to cows in the Resynch treatment. Treatment of Resynch cows with GnRH 21 d after AI did not affect fertility or pregnancy loss compared to Control cows (Table 2). Results from this study along with those of another study (Fricke et al., 2003) have resolved this initial controversy with using GnRH to pre-treat cows before pregnancy diagnosis for initiation of Resynch protocols, subsequently many dairies have adopted this strategy.

### Rapid Resynch

Follicular growth in high-producing dairy cows occurs as two follicular waves resulting in the presence of a dominant follicle capable of ovulation from about day 5 to 10 and day 16 to 21 of the estrous cycle (Ginther et al., 1996). In addition, a CL responsive to  $PGF_{2\alpha}$ -induced luteolysis is present in dairy cattle beginning about day 6 of the estrous cycle (Momont and Seguin, 1984). Assuming an estrous cycle duration of 21 to 23 d, cows should be at about day 5 to 7 of their estrous cycle when identified not-pregnant using US 28 d after an initial AI service, a time when most cows should have a CL responsive to  $PGF_{2\alpha}$ -induced luteolysis and a first-wave dominant follicle capable of ovulating in response to GnRH. Based on these assumptions, an abbreviated Resynch protocol in which the first GnRH injection is omitted and  $PGF_{2\alpha}$  is administered at the not-pregnant diagnosis using US 28 d after an initial TAI to induce regression of the CL with GnRH administered 2 d later to ovulate a dominant follicle followed by TAI should be feasible (Lucy et al., 2004). This strategy has been termed *rapid Resynch* and has been tested in several studies.

To test the rapid Resynch strategy, Stevenson et al. (2003) compared three rebreeding strategies in which nonpregnant cows that were not detected in estrus and reinseminated were diagnosed not-pregnant using US 27 to 29 d after a first service TAI. Not-pregnant cows from three commercial dairies were randomly assigned to receive either no further treatment until reinsemination after detected estrus (Control; n=189),  $PGF_{2\alpha}$  and reinsemination after detected estrus or TAI at 72 to 80 h after  $PGF_{2\alpha}$

Table 3. Reproductive performance of lactating dairy cows resynchronized after diagnosis of nonpregnancy 27 to 29 d after first-service TAI (adapted from Stevenson et al., 2003).

Item	Treatment					
	Control		PGF		PGF + GnRH	
	d or %	n	d or %	n	d or %	n
Days to reinsemination	55 ± 1	189	31 ± 2	108	31 ± 1	160
Conception rate	22.8	189	22.2	108	23.3	160
At estrus			27.2	81	33.3	9
TAI			7.4	27	23.2	151

treatment in the absence of estrus (PGF; n=108), or PGF<sub>2α</sub> followed by GnRH 48 h later and TAI 16 to 20 h after GnRH (PGF + GnRH; e.g., rapid resynch). Treated cows were reinseminated earlier (P<0.01; 31 ± 1 d) after first TAI than controls (55 ± 1 d), and fertility did not differ among the three treatments (Table 3). Similarly, Myer et al., (2004) conducted pregnancy diagnosis 28 d after TAI using US and administered PGF<sub>2α</sub> 29 d after TAI to nonpregnant cows followed by GnRH 48 d later and TAI. Cows (n=154) pre-treated with GnRH 22 d after TAI (Ovsynch) were compared to cows receiving rapid Resynch without GnRH pre-treatment. Fertility did not differ between the Ovsynch and rapid Resynch (24.6 vs. 23.9 %, respectively) treatments (Myer et al., 2004). Although the rapid Resynch strategy has merit, issues with the accuracy of early nonpregnancy diagnosis using US need to be considered before adopting a rapid Resynch strategy (see below).

### Effect of Timing of Initiation of Resynch after First Postpartum Timed AI on Fertility

A field trial was conducted to compare three intervals from first TAI to resynchronization of ovulation on a dairy incorporating US for early pregnancy diagnosis (Fricke et al., 2003). Lactating dairy cows (n=711) on a commercial dairy farm were enrolled into this study after Presynch + Ovsynch and TAI and were randomly assigned to each of three treatment groups for Resynch. All cows (n=235) in the first treatment (Day 19) received a GnRH injection 19 d after TAI and continued the Ovsynch protocol if diagnosed nonpregnant using US 26 d after TAI. Cows (n=240) in the second (Day 26) and cows (n=236) in the third (Day 33) treatments initiated Resynch if diagnosed not-pregnant using US 26 or 33 d after TAI, respectively. Resynch intervals for each of the three treatment groups were chosen to occur on Tuesdays so that injection schedules would remain consistent for all cows assigned to weekly breeding groups at any given time (Table 4). Implicit to the experimental design, first assessment of

pregnancy status was not conducted at the same interval after Presynch + Ovsynch and TAI among the three treatments. Pregnancy status after the first TAI was assessed 26 d after TAI for cows in the D19 and D26 treatments; whereas pregnancy status was assessed 33 d after TAI for cows in the D33 treatment. Overall pregnancies per AI (P/AI) to the first TAI was 40 % and was greater for D19 and D26 cows than for D33 cows (Table 5). This difference is likely due to a greater period in which embryonic mortality can occur in the D33 cows due to the increased interval from TAI to pregnancy diagnosis (26 vs. 33 d). When pregnancy status was reassessed for all treatments 68 d after TAI, overall P/AI was 31 % and did not differ among treatments (Table 5). Thus, differences in P/AI at the first pregnancy exam and pregnancy losses between the first and second pregnancy exams among treatments likely represent an artifact of time of assessment of pregnancy status after TAI inherent to the experimental design rather than to treatment differences. Overall P/AI to Resynch was 32 % and was greater for D26 and D33 cows than for D19 cows (Table 6). Thus, the most aggressive Resynch interval tested in this experiment resulted in unacceptably poor fertility compared to delaying Resynch by 7 to 14 d. Unfortunately, a direct comparison in fertility between the D26 and D33 treatments in this study was confounded by a 7-d difference in the interval to the first pregnancy diagnosis after Resynch TAI using US.

To further assess fertility between the D26 and D33 Resynch treatments, a follow-up study was conducted (Sterry et al., 2006). Lactating Holstein cows (n=763) at various days in milk and prior AI services were assigned randomly at TAI to receive the first GnRH injection of Resynch 26 (D26) or 33 (D33) d after TAI to resynchronize ovulation in cows failing to conceive. Cows in the D26 treatment received GnRH 26 d after TAI and continued Resynch only when diagnosed not-pregnant using US 33 d after TAI; whereas D33 cows initiated Resynch only when diagnosed not-pregnant using US 33 d after TAI. Cows were classified based on the

Table 4. Synchronization and resynchronization schedule for the D33 Resynch treatment (Fricke et al., 2003).

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Wk 1		PGF <sup>1</sup>				
Wk 2						
Wk 3		PGF				
Wk 4						
Wk 5		GnRH <sup>2</sup>				
Wk 6		PGF		GnRH		
Wk 7						
Wk 8						
Wk 9						
Wk 10						
Wk 11		GnRH				
Wk 12		PG <sup>3</sup> +PGF <sup>4</sup>		GnRH		

<sup>1</sup>PGF = prostaglandin F<sub>2α</sub>

<sup>2</sup>GnRH = gonadotropin-releasing hormone

<sup>3</sup>PG = pregnancy diagnosis.

<sup>4</sup>PGF only given to cows diagnosed not-pregnant.

presence or absence of a CL at the not pregnant diagnosis, and cows without a CL received a controlled internal drug releasing (CIDR) device during Resynch. When analyzed as a systematic strategy, fertility was greater for cows assigned to the D33 than the D26 Resynch treatment (39.4 vs. 28.6 %). A treatment by parity interaction was detected for P/AI after Resynch for not-pregnant cows with a CL in which primiparous cows had a greater P/AI than multiparous cows when Resynch was initiated 33 d after the initial TAI, and primiparous and multiparous cows when Resynch was initiated 26 d after the initial TAI. Interestingly, a similar effect of parity on

fertility of lactating Holstein cows (n=1079) to the D26 vs. D33 Resynch treatments was not detected in another study using a similar design but on a different farm (Silva et al., unpublished). Pregnancy loss for Resynch was 6.4 % from 33 to 40 d and 2.6 % from 40 to 61 d after Resynch TAI. Thus, delaying initiation of Resynch until 33 d after TAI increased P/AI for primiparous cows. Furthermore, pre-treating all cows with GnRH 33 d after TAI and delaying pregnancy diagnosis until 40 d after TAI would allow for action to be taken on the 6.4 % of cows that would be expected to experience pregnancy loss from 33 to 40 d after TAI.

Table 5. Pregnancies per artificial insemination (P/AI) and pregnancy loss after timed artificial insemination (TAI) to Ovsynch (adapted from Fricke et al., 2003).

Item	Treatment			Overall
	D19	D26	D33	
Interval from Ovsynch TAI to 1 <sup>st</sup> pregnancy exam (d)	26	26	33	-
P/AI at 1 <sup>st</sup> pregnancy exam, % (no./no.)	46 <sup>a</sup> (108/235)	42 <sup>a</sup> (101/240)	33 <sup>b</sup> (77/236)	40 (286/711)
Interval from Ovsynch TAI to 2 <sup>nd</sup> pregnancy exam (d)	68	68	68	-
P/AI at 2 <sup>nd</sup> pregnancy exam, % (no./no.)	33 (78/235)	30 (73/240)	29 (68/236)	31 (219/711)
Interval between pregnancy exams (d)	42	42	35	-
Pregnancy loss, % (no./no.)	28 <sup>a</sup> (30/108)	28 <sup>a</sup> (28/101)	12 <sup>b</sup> (9/77)	23 (67/286)

<sup>a,b</sup> Within a row, percentages with different superscripts differ ( $P < 0.01$ ) among treatments.

Table 6. Pregnancies per artificial insemination (P/AI) after timed artificial insemination (TAI) to Resynch beginning 19, 26, or 33 d after first TAI (adapted from Fricke et al., 2003).

Item	Treatment			Overall
	D19	D26	D33	
Mean ( $\pm$ SEM) interval (d) from Resynch TAI to pregnancy exam (range)	27.1 $\pm$ 0.4 (26 to 54)	26.6 $\pm$ 0.2 (26 to 40)	33.7 $\pm$ 0.4 (26 to 75)	-
P/AI, % (n)	23 <sup>a</sup> (120)	34 <sup>b</sup> (121)	38 <sup>b</sup> (143)	32 (384)

<sup>a,b</sup> Within a row, percentages with different superscripts differ ( $P < 0.01$ ) among treatments.

### Implications of Early Pregnancy Diagnosis and Pregnancy Loss for Resynch Strategies

The technology that makes early Resynch or the rapid Resynch system possible is US, a technology that has been adopted by bovine practitioners in certain regions of the U.S. Early pregnancy diagnosis improves reproductive performance by decreasing the interval between successive AI services and coupling a nonpregnancy diagnosis with an aggressive strategy to rapidly reinseminate these cows (Fricke, 2002). Although it has long been accepted that pregnancy status should be determined in dairy cows as soon as possible after AI, the accuracy of pregnancy diagnosis outcomes determined early after AI are confounded by subsequent pregnancy loss (Studer, 1969; Melrose, 1979). Recent research on the practical implementation of early pregnancy diagnosis using US in a systematic synchronization and resynchronization system has confirmed the notion that pregnancy diagnosis using US can be conducted too early and illustrates the pitfalls and limitations of early pregnancy diagnosis in lactating dairy cows (Fricke et al., 2003). Pregnancy loss diminishes the benefit of early pregnancy diagnosis in two ways. First, because of the high rate of pregnancy loss that occurs early, the magnitude of pregnancy loss detected is greater the earlier after TAI that a positive diagnosis is made. Thus, the

earlier that pregnancy is diagnosed after TAI, the fewer nonpregnant cows are identified to which a management strategy can be implemented to resynchronize them. Second and more important, cows diagnosed pregnant earlier after TAI have a greater risk for subsequent pregnancy loss compared to cows diagnosed later after TAI. If left unidentified, cows diagnosed pregnant early after TAI that subsequently lose that pregnancy reduce reproductive efficiency by extending the interval from calving to the conception that results in a full-term pregnancy.

The accuracy of pregnancy outcomes using US after TAI was assessed in a field trial (Silva et al., 2006). Pregnancy examinations were performed by one herd veterinarian throughout the study using US in lactating Holstein cows ( $n=877$ ) 27 d after first postpartum TAI. Outcomes were categorized as: pregnant (PG) = CL, normal uterine fluid, embryo visualized; questionable pregnant 1 (QP1) = CL, normal uterine fluid, embryo not visualized; questionable pregnant 2 (QP2) = CL, abnormal uterine fluid, embryo not visualized; pregnancy loss (PL) = nonviable embryo; nonpregnant (NP) = no CL and/or uterine fluid. Outcomes using US were compared to those categorized PG or NP using a pregnancy-associated glycoprotein (PAG) ELISA of plasma samples collected at US (Table 7). Outcomes for cows in which US and PAG agreed were considered correct; whereas cows in which outcomes

Table 7. Accuracy of transrectal ultrasonography (US) scanning scores assessed 27 and 39 d after a timed AI (TAI) (adapted from Silva et al., 2006).

US Score	Accuracy 27 d after TAI		Accuracy 39 d after TAI	
	Frequency, % (n)	Incorrect, % (n)	Frequency, % (n)	Incorrect, % (n)
PG	17.3 (1903)	2.7 (328)	47.8 (1321)	0.1 (631)
QP1	19.9 (1903)	10.0 (372)	1.3 (1321)	5.9 (17)
QP2	3.4 (1903)	56.7 (60)	0.0 (0)	0.0 (0)
PL	0.6 (1903)	18.2 (11)	1.5 (1321)	0.0 (16)
NP	58.7 (1903)	1.9 (1112)	49.4 (1321)	0.3 (644)

Table 8. Pregnancies per AI (P/AI) 66 d after timed AI (TAI) to a Resynch protocol initiated 32 d after first TAI (Resynch) or a presynchronized Resynch (Presynch + Resynch) protocol (Silva et al., unpublished).

Treatment				Treatment	P	
Resynch		Presynch + Resynch			Parity	Interaction
Primiparous	Multiparous	Primiparous	Multiparous			
23.8	25.2	35.0	31.6	0.02	0.69	0.50

disagreed were rechecked using US 32 d after TAI. These results demonstrate that although agreement between PAG and US at 27 d after TAI was acceptable, US outcomes of QP1, QP2 and PL (23.9% of all US outcomes 27 d after TAI) were less accurate than PG or NP outcomes. Based on these results, early pregnancy diagnosis outcomes using US 27 d after TAI was less accurate than US 39 d after TAI.

### Optimization of Resynch Protocols

Because fertility to Resynch was poor for cows lacking a CL at the first GnRH or PGF<sub>2α</sub> injections of Resynch compared to those cows with a CL at these times (Fricke et al., 2003), alternative treatments aimed at improving fertility of cows based on their stage of the cycle at initiation of Resynch may further improve an overall resynchronization strategy. To optimize fertility to Resynch TAI, Bartolome et al. (2005) assigned cows to targeted Resynch treatments according to the estimated stage of the estrous cycle (e.g., diestrus, metestrus, proestrus, anovular, or cystic) at a not-pregnant diagnosis based on US and palpation 30 d after AI (d 0). Cows in diestrus were resynchronized using Resynch (n=156) or Modified Quicksynch (PGF<sub>2α</sub>, d 0; estradiol cypionate [ECP], d 1; AI at detected estrus [AIDE], d 2; and Ovsynch on d 4 if not detected in estrus; n=142); whereas cows in metestrus were resynchronized using Resynch (n=68), Heatsynch (GnRH, d 0; PGF<sub>2α</sub>, d 7; ECP, d 8; AIDE, d 9; or TAI, d 10; n=62), or GnRH + Resynch (GnRH, d 0; Resynch, d 8; n=64). For diestrus cows, P/AI 55 d after AI was similar for Resynch (24 %) and Modified Quicksynch (26 %) cows. For metestrus cows, P/AI 55 d after AI were greater for GnRH + Resynch (25 %) than for Heatsynch (13 %). For cows with ovarian cysts (n=97), P/AI 55 d after AI was greater for GnRH + Resynch (27 %) than for Resynch (19 %). Thus, assignment of Resynch treatments based on the estimated stage of the estrous cycle or the presence of ovarian cysts improved fertility in this study.

Another strategy to optimize fertility to Resynch and TAI has been to determine the optimal interval after TAI to initiate Resynch based on assumptions

regarding the physiology of the estrous cycle (Fricke et al., 2003; Sterry et al., 2006). Assuming an estrous cycle duration of 21 to 23 d, initiation of Resynch 32 to 33 d after TAI should ensure that the first GnRH injection of Resynch occurs between Day 5 to 12 of the estrous cycle, a stage of the cycle when a CL should be present and that results in greater fertility when Ovsynch is initiated (Vasconcelos et al., 1999; Moreira et al., 2000a). Despite this logic, 16 % to 22 % of cows lack a CL 33 d after TAI (Fricke et al., 2003; Sterry et al., 2006) suggesting that there is significant *biological drift* among a group of cows at various times after synchronization using Presynch + Ovsynch and TAI. Reasons for this biological drift among cows include normal variation in estrous cycle duration, the incidence of pregnancy loss greater than 24 d after TAI and subsequent return to estrus, and/or lack of synchrony to Presynch + Ovsynch.

Because of this biological drift after an initial TAI among groups of cows, an alternative approach might be to presynchronize cows before initiation of Resynch. In a preliminary experiment (Silva et al., unpublished), cows diagnosed not-pregnant to a prior TAI service (n=593) were randomly assigned to each of two Resynch treatments. Cows diagnosed not-pregnant 31 d after TAI in the first treatment received the first GnRH injection of Resynch 32 d after the prior TAI service (Resynch). Cows diagnosed not-pregnant using US 31 d after TAI in the second treatment were presynchronized using a single injection of PGF<sub>2α</sub> 34 d after the prior TAI, and then received the first GnRH injection of Resynch 12 d later (Presynch + Resynch). Preliminary results show an increase in fertility to Resynch TAI due to presynchronization for both primiparous and multiparous cows (Table 8). Interestingly, this presynchronization effect on fertility to Resynch TAI is similar in magnitude to that reported for Presynch + Ovsynch (Moreira et al., 2001; Navanukraw et al., 2004). Although Presynch + Resynch improved fertility to TAI compared to a standard Resynch interval of 32 d, the interval between TAI was increased by 14 d. Further work is needed to determine whether presynchronization improves 21-d pregnancy rates compared to the standard Resynch interval of 32 d after TAI.

## Protocol Compliance

Both scientific research and anecdotal evidence supports the idea that systematic synchronization and resynchronization systems are viable management alternatives for dairy cows managed in confinement-based dairy systems. Many factors affect reproductive performance, and many consultants have observed a wide range of performance among farms that have adopted the exact same protocols. Poor performance of these protocols is rarely due to physiologic responses of individual cows, but often can be attributed to protocol compliance issues at the farm level. To achieve success, each farm has to develop a system to administer the correct injections to the correct group of cows on the correct days, then subsequently AI the correct group of cows. A standard Presynch + Ovsynch protocol for submitting cows for first AI service requires that each individual cow receive 5 consecutive injections at the appropriate time and in the correct sequence. Failure to administer any one of these 5 injections or administration in an incorrect sequence will reduce the conception risk to TAI and ultimately will result in a delay in establishing pregnancy. For a farm that achieves an injection protocol accuracy of 95 % on any given injection day (e.g., 95 % of the cows that should get an injection actually get the correct one), on average nearly one in four cows will not successfully complete the 5 injections of the Presynch + Ovsynch protocol (e.g.,  $0.95 \times 0.95 \times 0.95 \times 0.95 \times 0.95 = 0.77$ ). Thus, farms that cannot achieve acceptable protocol compliance should consider focusing on other methods to improve AI service risk.

## Conclusions

Although coupling a nonpregnancy diagnosis with a management decision to quickly reinstate AI service may improve reproductive efficiency by decreasing the interval between AI services, early pregnancy loss and the effectiveness of Resynch initiated at certain physiologic stages post breeding may limit the effectiveness of the early Resynch and rapid Resynch strategies tested thus far. Thus, a justifiable Resynch strategy is to pre-treat all cows with GnRH 7 d before pregnancy diagnosis 32 to 33 d after TAI, identify cows failing to conceive to TAI and administer PGF<sub>2α</sub> to cows diagnosed not-pregnant 39 to 40 d after TAI and complete the Resynch protocol. This recommendation is based on data in which the earliest Resynch intervals of 19 or 26 d after TAI do not yield the greatest fertility (Fricke et al., 2003; Sterry et al., 2006) and the notion that assessment of pregnancy status should be delayed until the latest possible time after TAI and during Resynch to ensure

that diagnostic outcomes using US are not confounded by subsequent pregnancy loss (Silva et al., 2006). A practical advantage of the 32 to 33 d Resynch interval is that it results in an even number of weeks between TAI and therefore is easily implemented in herds that perform pregnancy diagnosis every other week. Finally, although presynchronization increased fertility to Resynch and TAI, further work is needed to determine whether this strategy improves 21-d pregnancy rates compared to the recommended Resynch interval of 32 to 33 d after TAI.

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