Strategies for Resynchronization of Ovulation and Timed AI

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Introduction

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 (Caraviello et al., 2006). 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.

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 1).

Table 1. Synch and Resynch schedule for the D33 Resynch treatment (Fricke et al., 2003).

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

PGF = prostaglandin $F_{2\alpha}$, GnRH = gonadotropin-releasing hormone, PG = pregnancy diagnosis.

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 P/AI to the first TAI was 40% and was greater for D19 and D26 cows than for D33 cows (Table 2). 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 3). 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.

Table 2. Pregnancies per artificial insemination (P/AI) and pregnancy loss after timed artificial insemination (TAI) to Ovsynch (adapted from Fricke et al., 2003).

	Treatment			
Item	D19	D26	D33	Overall
Interval from Ovsynch TAI to 1st pregnancy exam (d)	26	26	33	-
P/AI at 1st pregnancy exam, % (no./no.)	46[a] (108/235)	42[a] (101/240)	33[b] (77/236)	40 (286/711)
Interval from Ovsynch TAI to 2nd pregnancy exam (d)	68	68	68	-
P/AI at 2nd 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, %	28[a]	28[a]	12[b]	23
(no./no.)	(30/108)	(28/101)	(9/77)	(67/286)

[a,b]Within a row, percentages with different superscripts differ (*P*<0.01) among treatments.

Table 3. 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).

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

[a,b]Within a row, percentages with different superscripts differ (*P*<0.01) among treatments.

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 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., 2009). 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.

Optimization of Resynch Protocols

Because fertility to Resynch was poor for cows lacking a CL at the first GnRH or $PGF_{2\alpha}$ 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 resychronization 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 ($PGF_{2\alpha}$, 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; $PGF_{2\alpha}$, 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.

Presynchronization before Initiation of Resynch using Double Ovsynch

An alternative approach to improve fertility to resynchronized breedings might be to presynchronize cows before initiation of Resynch. A field trial was conducted (Giordano et al., 2012) to determine if using a Double-Ovsynch protocol (DO; Pre-Resynch: GnRH-7 d-PGF2α-3 d-GnRH, 7 d later Breeding-Resynch: GnRH-7 d-PGF2α-56 h-GnRH-16 h-TAI) to resynchronize ovulation after a previous timed artificial insemination (TAI) would increase synchrony and pregnancies per AI (P/AI) compared to an Ovsynch protocol initiated 32 d after TAI (D32; GnRH-7 d-PGF2α-56 h-GnRH-16 h-TAI). All DO cows received the first GnRH injection of Pre-Resynch 22 d after TAI, and cows (n = 981) diagnosed not pregnant using transrectal ultrasonography 29 d after TAI continued the protocol. Pregnancy status for all D32 cows was evaluated 29 d after TAI so fertility and pregnancy loss could be compared to DO cows. All D32 cows received the first GnRH injection of Ovsynch 32 d after TAI, and cows (n = 956) diagnosed not pregnant using transrectal palpation 39 d after TAI continued the protocol.

Overall, P/AI 29 d after TAI was not affected by parity and was greater for DO vs. D32 cows (Table 4). Pregnancy loss from 29 to 74 d after TAI was not affected by parity or treatment. The percentage of cows with a functional CL (P4 $\geq$ 0.5 ng/mL) at GnRH1 was greater for DO than D32 cows (86.7 vs. 62.5%) with most DO cows having medium P4 (73%; 0.5 to 3.49 ng/ml), whereas, most D32 cows had either low (37%; < 0.5 ng/mL) or high (36%; > 3.5 ng/ml) P4 at GnRH1. In a subgroup of cows from each treatment, ultrasonography (n = 751) and serum progesterone (P4) concentrations (n = 743) were used to determine the presence of a functional corpus luteum (CL) and ovulation to the first GnRH injection of D32 and Breeding-Resynch of DO (GnRH1), luteal regression after PGF before TAI, and ovulation to the GnRH injection before TAI (GnRH2).

Table 4. Pregnancies per AI (P/AI) at 29, 39, and 74 d after TAI, and pregnancy loss from 29 to 39, 39 to 74, and 29 to 74 d after TAI for cows resynchronized using Ovsynch initiated 32 d after a previous TAI (D32) or Double-Ovsynch (DO). Adapted from Giordano et al., 2012.

	Treatment		
Item	D32	DO	*P*-value
P/AI 29 d, % (n/n)	30.0 (287/956)	38.7 (380/981)	
LS Means ± SEM	29.7 ± 1.0	38.2 ± 2.0	<0.0001
P/AI 39 d, % (n/n)	27.0 (258/955)	35.3 (345/977)	
LS Means ± SEM	26.7 ± 1.0	34.8 ± 2.0	0.0001
P/AI 74 d, % (n/n)	24.5 (233/952)	32.9 (320/972)	
LS Means ± SEM	24.2 ± 2.0	32.5 ± 1.5	<0.0001
Loss 29 to 39 d, % (n/n)	9.8 (28/286)	8.2 (31/376)	
LS Means ± SEM	9.8 ± 1.8	8.2 ± 1.4	0.62
Loss 39 to 74 d, % (n/n)	8.6 (22/255)	5.9 (20/340)	
LS Means ± SEM	6.8 ± 2.0	6.1 ± 1.0	0.76
Loss 29 to 74 d, % (n/n)	17.7 (50/283)	13.8 (51/371)	
LS Means ± SEM	16.3 ± 2.0	14.0 ± 2.0	0.44

Table 5 shows that a greater percentage of DO cows were synchronized compared to D32 cows (71.8 vs. 50.5%) primarily due to a greater percentage of D32 than DO cows without a functional CL at the PGF injection before TAI (34.8 vs. 17.0%) or without complete CL regression before GnRH2 (16.8 vs. 6.5%).

Table 5. Synchronization rate for cows resynchronized using Ovsynch initiated 32 d after a previous TAI (D32) or Double-Ovsynch (DO). Adapted from Giordano et al., 2012.

	Treatment		
	D32 % (n/n)	DO % (n/n)	P-value
P4 at time of PGF			
Cows with Low P4	34.8 (73/210)	17.0 (38/233)	<0.0001
P/AI for cows with Low P4	9.6 (7/73)	5.3 (2/38)	0.44
P/AI for cows with High P4	35.8 (49/137)	37.8 (70/185)	0.70
P4 at time of GnRH2			
Cows with High P4	16.8 (23/137)	6.5 (12/185)	0.005
P/AI for cows with High P4	13.0 (3/23)	8.3 (1/12)	0.68
P/AI for cows with Low P4	40.4 (46/114)	39.9 (69/173)	0.94
Ovulation to GnRH2			
Cows with no ovulation	7.0 (8/114)	7.5 (13/173)	0.87
P/AI for cows with no ovulation	0.0 (0/8)	0.0 (0/13)	1.00

P/AI for cows with ovulation	43.4 (46/106)	43.1 (69/160)	0.97
Synchronization Rate			
Synchronized cows	50.5 (106/210)	71.8 (160/223)	0.0001
P/AI for cows not synchronized	9.6 (10/104)	4.8 (3/63)	0.27
P/AI for synchronized cows	43.4 (46/106)	43.1 (69/160)	0.97

Based on the results from Giordano et al. (2011), we conclude that DO increased fertility of lactating dairy cows during a resynchronization program primarily by increasing synchronization of cows during the Ovsynch protocol before TAI.

Effect of Timing of Initiation of Resynch and Presynchronization with GnRH

A field trial was conducted to assess the effect of timing of initiation of Resynch and presynchronization with GnRH on fertility of Resynch breedings (Lopes et al., 2011). Lactating Holstein cows (n = 1,456) were randomized to a 2x2 factorial design (Figure 1) resulting in four Resynch treatments: 1) Ovsynch (GnRH–7 d–PGF–56 h–GnRH–16 h–TAI) initiated d 32 ± 3 d after AI (GPG 32); 2) presynchronization with 100 µg GnRH 25 ± 3 d after AI and Ovsynch initiated 32 ± 3 d after AI at nonpregnancy diagnosis (GGPG 32); 3) Ovsynch initiated 39 ± 3 d after AI (GPG 39); 4) presynchronization with 100 µg GnRH 32 ± 3 d after AI at nonpregnancy diagnosis and Ovsynch initiated 39 ± 3 d after AI (GGPG 39).

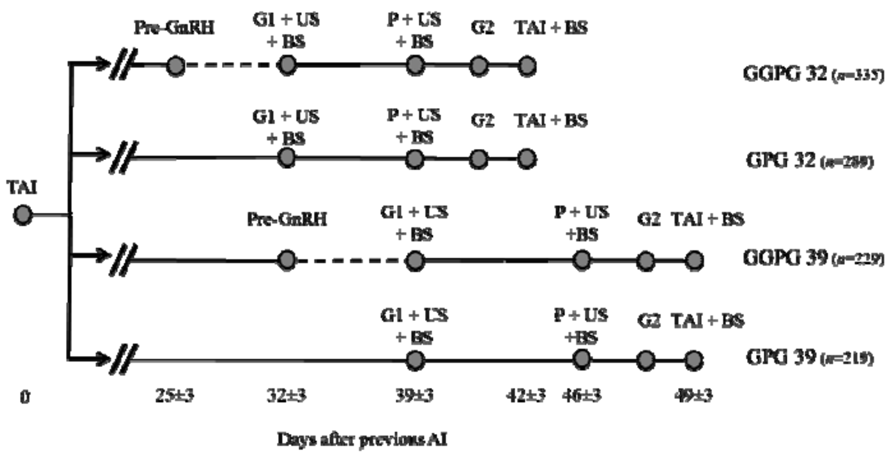

Figure 1. Resynchronization protocols for cows assigned randomly to initiation of resynch 32 ± 3 d after previous AI with presynchronization with GnRH (GGPG 32), initiation of resynch 32 ± 3 d after previous AI with no presynchronization with GnRH (GPG 32), initiation of resynch 39 ± 3 d after previous AI with presynchronization with GnRH (GGPG 39) and initiation of resynch 39 ± 3 d after previous AI with no presynchronization with GnRH (GPG 39) for second and subsequent postpartum TAI services. Cows received an initial TAI after synchronization of ovulation Ovsynch or after estrous detection. Every week, cohorts of cows were randomly assigned to one of four treatments for resynchronization of ovulation and TAI for second and greater services. Blood samples (BS) were collected at G1 to determine progesterone concentrations from all cows. In a subgroup of cows ovarian ultrasonography (US) was performed at the time of the first GnRH (G1) and $PGF_{2\alpha}$ of resynch and blood samples (BS) were collected at G1 to determine progesterone concentrations and then at the time of $PGF_{2\alpha}$ and TAI to determine luteal regression after $PGF_{2\alpha}$ administration.

Overall, 344 cows were inseminated to estrus between enrollment (25 ± 3 d after AI) and TAI of Resynch treatments, and 1,112 cows (GPG 32 = 289, GGPG32 = 335, GPG39 = 219 and GGPG 39 = 269) received TAI. Blood samples were collected from all cows at the first GnRH injection of Resynch (G1), and ovarian structures were evaluated and blood samples were collected at G1, PGF, and TAI of the Resynch protocols in a subgroup of cows (GPG 32 = 100, GGPG 32 = 115, GPG 39 = 93 and GGPG 39 = 109). Pregnancies per AI (P/AI) 32 d after AI were not affected by parity and did not differ among treatments. When analyzed as main effects, presynchronization with GnRH increased P/AI (33.8% vs. 38.9% for GPG vs. GGPG cows), whereas timing of initiation of Resynch did not. Presynchronization with GnRH increased the proportion of cows with high P4 (> 0.5 ng/mL) at G1, and cows with high P4 at G1 had greater P/AI than cows with low P4 (38.5% vs. 30.0%). Ovulation to G1 decreased luteal regression after PGF (87.3% vs. 78.5%), and high P4 at G1 decreased ovulation to G1 (69.1% vs. 40.9%). We conclude that presynchronization with GnRH 7 d before initiation of resynchronization increased fertility of dairy cows whereas timing of initiation of resynchronization did not.

Table 6. Effect of presynchronization with GnRH 7 d before initiation of Resynch on percentage of lactating dairy cows with their estrous cycle synchronized. Adapted from Lopes et al., 2011.

	Treatment		
	GPG % (n/n)	GGPG % (n/n)	*P*-value
P4 at time of $PGF_{2\alpha}$			
Cows with P4 < 1 ng/mL	31.6 (61/193)	20.0 (45/224)	0.007
P/AI for cows with P4 < 1 ng/mL	32.8 (20/61)	26.7 (12/45)	0.53
P/AI for cows with P4 ≥ 1 ng/mL	41.6 (55/132)	44.1 (79/179)	0.63
P4 at TAI			
Cows with P4 ≥ 0.3 ng/mL	18.9 (25/132)	16.7 (30/179)	0.60
P/AI for cows with P4 ≥ 0.3 ng/mL	8.0 (2/25)	13.3 (4/30)	0.97
P/AI for cows with P4 < 0.3 ng/mL	49.5 (53/107)	50.3 (75/149)	0.86
Synchronization Rate			
Synchronized cows	55.4 (107/193)	66.5 (149/224)	0.02
P/AI Not Synchronized	25.6 (22/86)	21.3 (16/75)	0.86
P/AI Synchronized	49.6 (53/107)	50.3 (75/149)	0.52

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 x 0.95 x 0.95 x 0.95 x 0.95 = 0.77). Thus, farms that cannot achieve acceptable protocol compliance should consider focusing on other methods to improve AI service risk.

Conclusions

A proven Resynch strategy is to pre-treat all cows with GnRH 7 d before pregnancy diagnosis 32 d after TAI, identify cows failing to conceive to TAI and administer $PGF_{2\alpha}$ to cows diagnosed not-pregnant 39 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). Fertility to a Day 32 Resynch, however, are lower than that observed to first service protocol in which presynchronization is used. Results from Lopes et al. (2011) and Giordano et al. (2012) demonstrate that presynchronization before initiation of Resynch can increase fertility to Resynch breedings. Currently, we are investigating strategies to improve fertility to Resynch breedings but reduce the interbreeding interval.

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