Fertility Programs for First AI

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Introduction

Hormonal synchronization protocols have been incorporated widely into reproductive management programs by dairy farmers (Caraviello et al., 2006; Norman et al., 2009). The initial impact of TIMED AI protocols on 21-day pregnancy rates in U.S. dairy herds has been to increase the AI service rate (Norman et al., 2009); however, a deeper understanding of the physiology underlying the Ovsynch protocol has allowed for a dramatic increase in fertility to timed AI. Thus, the newest iteration of synchronization protocols are perhaps better described as fertility programs for high-producing lactating dairy cows.

Ovsynch and Timed AI

A long-standing goal of reproductive biologists was to develop a hormonal synchronization protocol that would allow for a timed AI to overcome limitations associated with visual detection of estrus thereby increasing the AI service rate. This goal was realized in 1995 with development and publication of the Ovsynch protocol, a synchronization protocol in which three sequential hormonal treatments are used to control ovarian cycles (Pursley et al., 1995). The initial GnRH treatment of an Ovsynch protocol is referred to as **G1** and the final GnRH treatment of an Ovsynch protocol immediately preceding timed AI is referred to as **G2**.

In the first field trial that evaluated the Ovsynch protocol for reproductive management (Pursley et al., 1997), lactating dairy cows managed using only the Ovsynch protocol and timed AI without detection of estrus had fewer median days to first AI (54 vs. 83) and fewer days open (99 vs. 118) than cows inseminated to estrus, whereas P/AI to first AI was similar (37% vs. 39% for timed AI vs. estrus, respectively) even though cows managed using the Ovsynch protocol and timed AI were inseminated earlier postpartum. Thus, the initial impact of adoption of the original Ovsynch protocol by dairy farms was to increase the 21-day pregnancy rate by increasing the AI service rate while maintaining fertility.

Presynchronization using $PGF_{2\alpha}$

The first attempt to increase fertility to timed AI was to “presynchronize” cows before initiation of an Ovsynch protocol. Because initiation of the Ovsynch protocol between days 5 to 12 of the estrous cycle resulted in increased fertility than earlier or later start of the Ovsynch protocol during the estrous cycle (Vasconcelos et al., 1999), a presynchronization strategy was tested using two $PGF_{2\alpha}$ treatments administered 14 d apart with the second $PGF_{2\alpha}$ treatment preceding G1 by 12 d (Moreira et al., 2001; Table 1). When only cycling cows were included in the statistical analysis, fertility to timed AI increased from 29% for cows submitted to an Ovsynch protocol to 43% for cows submitted to a Presynch-Ovsynch protocol; however, no statistical difference was detected when all cows (cycling and anovular) were included in the analysis.

Many farms initially implemented a Presynch-Ovsynch protocol using a 14 d interval between the second $PGF_{2\alpha}$ treatment of Presynch and G1 of the Ovsynch protocol to keep the first 4 treatments of the Presynch-Ovsynch protocol on the same day of the week. Decreasing the interval between the second $PGF_{2\alpha}$ treatment of Presynch to G1 from 14 to 11 d, however, increased ovulatory response to G1 and improved fertility by approximately 7 percentage points when all cows were submitted to TAI (Galvão et al., 2007). Initiation of G1 14 d after the second $PGF_{2\alpha}$ treatment of Presynch decreases fertility to timed AI because of a concomitant decrease in ovulatory response to G1 (Galvão et al., 2007) due to circulating progesterone levels and the pattern of follicular waves in high-producing cows. These data support a decreased interval (i.e., 10 to 12 d rather than 14 d) between the second $PGF_{2\alpha}$ treatment of Presynch and G1 of the Ovsynch protocol when 100% of cows are submitted for first timed AI.

Table 1. Hormone treatment and timed artificial insemination schedule for the Presynch-Ovsynch protocol based on the results of Moreira et al., 2001.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
			PGF			
			PGF			
	GnRH					
	PGF		GnRH	TAI		

PGF = prostaglandin $F_{2\alpha}$, GnRH = gonadotropin-releasing hormone, TAI = timed artificial insemination.

Combining Activity Monitors with Timed AI Protocols

Despite the widespread adoption of hormonal synchronization protocols that allow for timed AI, detection of behavioral estrus continues to play an important role in the overall reproductive management program on most dairies in the U.S. (Caraviello et al., 2006; Miller et al., 2007). A combined approach in which AI is based both on activity detected by an activity monitoring system followed by submission of cows not detected with activity to timed AI after synchronization of ovulation may be an effective and economical strategy to submit lactating dairy cows for first AI.

Table 2. Effect of treatment on percentage of lactating Holstein cows with activity based on an activity monitoring system, and pregnancies per AI (P/AI) for cows with or without activity and inseminated to activity (AI) or inseminated after synchronization of ovulation (TAI). Adapted from Fricke et al., 2014.

	Treatment	
Item	1	2
Cows with detected activity, % (n/n)	69 (230/335)	70 (232/331)
P/AI 35 d after AI, % (n/n)		
Cows with activity receiving AI	30[a] (68/230)	-
Cows with activity receiving TAI	-	41[b] (96/232)
Cows with no activity receiving TAI	36 (37/104)	35 (35/99)
Overall P/AI 35 d after AI, % (n/n)	32[c] (105/333)	40[d] (131/331)
P/AI 67 d after AI, % (n/n)		
Cows with activity receiving AI	27 (62/230)	-
Cows with activity receiving TAI	-	40 (92/232)
Cows with no activity receiving TAI	33 (34/104)	35 (35/99)
Overall P/AI 67 d after AI, % (n/n)	29[c] (96/333)	38[d] (127/331)

[a,b]Within a treatment by activity subgroup, statistical contrast differed (P = 0.004).
[c,d]Within a row, percentages with different superscripts differed (P = 0.0454).
[1]Treatments were: 1) cows inseminated based on an activity monitoring system after a presynchronization protocol with cows not detected with activity receiving TAI after synchronization of ovulation using an Ovsynch protocol; 2) cows receiving TAI after a Presynch-Ovsynch protocol.

We conducted a field trial to compare reproductive performance of lactating dairy cows managed for first AI using timed AI with or without detection of estrus using an activity monitoring system (Fricke et al., 2014). All cows were submitted to a Presynch-Ovsynch protocol (Table 1), and activity was monitored in all cows using an activity monitoring system (Heatime, SCR Engineers Ltd., Netanya, Israel) beginning at 24 ± 3 DIM. Cows in treatment 1 with increased activity after the second $PGF_{2α}$ injection were inseminated based on activity, whereas cows without increased activity were submitted to an Ovsynch protocol beginning 12 d after the second $PGF_{2α}$ treatment of the presynchronization protocol and received a timed AI at 75 ± 3 days in milk. Cows in treatment 2 with increased activity after the second $PGF_{2α}$ treatment were recorded by the activity monitoring system software but were not inseminated so that all cows in treatment 2 completed the Presynch-Ovsynch protocol and received a timed AI at 75 ± 3 days in milk regardless of whether or not they were detected with increased activity after the second $PGF_{2α}$ treatment.

The activity monitoring system detected increased activity in 69% and 70% of cows after the second $PGF_{2α}$ injection in treatments 1 and 2, respectively (Table 2) which is about 10 to 15 percentage points greater than that reported in studies using tail chalk after the second $PGF_{2α}$ injection of a Presynch-Ovsynch protocol (Stevenson and Phatak, 2005; Chebel and Santos, 2010). Overall, cows in treatment 1 in which inseminations occurred as a combination between AI to activity and timed AI had fewer P/AI compared to cows in treatment 2 in which all cows received timed AI after completing the Presynch-Ovsynch protocol (Table 2). The reduction in P/AI due to inseminating cows with increased activity after the second $PGF_{2α}$ injection was expected because the increase in P/AI due to presynchronization with $PGF_{2α}$ likely results from synchronizing estrus after the second $PGF_{2α}$ injection (Navanukraw et al., 2004) so most cows initiate the Ovsynch protocol on days 5 to 9 of the ensuing estrous cycle thereby improving P/AI to timed AI (Vasconcelos et al., 1999). Inseminating 70% of cows based on activity after the second $PGF_{2α}$ injection removed the presynchronized cows from the protocol thereby negating the increase in P/AI due to presynchronization. Cows without increased activity after the second $PGF_{2α}$ injection and submitted to an Ovsynch protocol had P/AI of 33% and 35% for treatments 1 and 2, respectively (Table 2). Pregnancy outcomes of anovular cows subjected to an Ovsynch protocol is generally about 20% compared to about 35% for cycling cows starting an Ovsynch protocol at a random stage of the cycle (Gümen et al., 2003; Stevenson et al., 2008). Thus, cows without activity that received an Ovsynch protocol had a P/AI similar to that of cycling cows starting an Ovsynch protocol at a random stage of the cycle. Thus, aggressive submission of cows to an Ovsynch protocol after failing to be detected with increased activity is an effective management strategy to establish pregnancy in this subgroup of cows. Results from Fricke et al. (2014) support a management strategy in which the 30% of cows not detected with activity are aggressively submitted to an Ovsynch protocol rather than continuing to detect activity using an activity monitoring system.

Fertility Programs for Lactating Dairy Cows

Even though the Presynch-Ovsynch protocol was originally developed to increase the fertility of cows submitted to TAI, many farms inseminate cows detected in estrus after the second $PGF_{2α}$ treatment of a Presynch-Ovsynch protocol followed by submission of cows not detected in estrus to an Ovsynch-56 protocol as described in the previous experiment. A recent meta-analysis, however, supports the idea that incorporation of AI to estrus into a Presynch-Ovsynch protocol decreases P/AI for cows not detected in estrus and submitted to timed AI than when all cows were allowed to complete the protocol and receive timed AI (Borchardt et al., 2016). This decrease in P/AI to timed AI occurs because cycling cows that would have been presynchronized so that G1 occurred at an optimal time of the estrous cycle are removed from the protocol after AI to estrus thereby negating the presynchronization effect. Further, anovular cows submitted to a Presynch-Ovsynch protocol have decreased P/AI than their cycling herd mates. Because anovular cows lack a CL and therefore do not respond to the two initial $PGF_{2α}$

treatments of a Presynch-Ovsynch protocol, G1 occurs in a low progesterone environment resulting in fewer P/AI to timed AI. Because anovular cows represent nearly 1 in 4 cows submitted for first timed AI, presynchronization strategies using $PGF_{2\alpha}$ alone with or without inclusion of detection of estrus do not yield high P/AI to Timed AI.

Fertility Programs that Combine $PGF_{2\alpha}$ and GnRH for Presynchronization

Two limitations of presynchronization strategies that use $PGF_{2\alpha}$ alone (e.g., Presynch-Ovsynch) are that 1) $PGF_{2\alpha}$ does not affect anovular cows or resolve the anovular condition before G1 of the Ovsynch protocol, and 2) follicular growth is not tightly synchronized after two sequential $PGF_{2\alpha}$ treatments administered 14 d apart. By contrast, presynchronization strategies that combine GnRH and $PGF_{2\alpha}$ overcome both of these limitations thereby increasing fertility to timed AI. For example, when an Ovsynch protocol was used to presynchronize cows before G1 of an Ovsynch protocol (i.e., a Double-Ovsynch protocol), cows submitted to the Double-Ovsynch protocol had more P/AI than cows submitted to a Presynch-Ovsynch protocol (50% vs. 42%; Souza et al., 2008). Because of the inclusion of GnRH, presynchronization strategies that combine GnRH and $PGF_{2\alpha}$ increase P/AI to timed AI by resolving the anovular condition before initiation of G1, by more tightly controlling follicular development and luteal regression, and by presynchronizing cows so that G1 occurs on either day 6 or 7 of the estrous cycle, which optimizes the response of cows to each sequential treatment of the Ovsynch protocol.

Several experiments have assessed the effect of an additional $PGF_{2\alpha}$ treatment within a Double-Ovsynch protocol to decrease progesterone at G2 (Brusveen et al., 2009; Wiltbank et al., 2015). Adding a second $PGF_{2\alpha}$ treatment 24 h after the first $PGF_{2\alpha}$ treatment during the second Ovsynch portion of a Double-Ovsynch protocol for first timed AI dramatically increased the proportion of cows undergoing complete luteal regression thereby increasing the proportion of pregnant primiparous cows by 4.6% and the proportion of pregnant multiparous cows by 23% (Wiltbank et al., 2015). Thus, the treatment by parity effect on P/AI to timed AI after a Double-Ovsynch protocol discussed previously can at least partially be explained by a lack of luteal regression in multiparous cows that can be overcome by adding a second $PGF_{2\alpha}$ before G2 of the protocol.

Taken together, these data support that ~50% of cows become pregnant to first timed AI after a modified Double-Ovsynch protocol (Table 3). The advantages of using 100% timed AI after a modified Double-Ovsynch protocol for first timed AI include precise control of the interval from calving to first AI such that all cows in the herd receive timed AI within 7 d of the end of the VWP (in herds in which timed AI is conducted weekly) and yielding exceptionally high fertility to first timed AI. When optimized, these two factors dramatically increase the 21-d pregnancy rate. Presynchronization strategies that combine GnRH and $PGF_{2\alpha}$ to resolve anovular conditions and optimize timing of initiation of G1 and addition of a second $PGF_{2\alpha}$ treatment 24 h after the first $PGF_{2\alpha}$ treatment within an Ovsynch protocol currently represents the most aggressive method for submitting cows for first AI while also yielding high P/AI to timed AI.

Table 3. Hormone injection and timed artificial insemination schedule for the Double-Ovsynch protocol based on the results of Wiltbank et al., 2015.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
					GnRH	
					PGF	
	GnRH					
	GnRH					

	PGF	PGF	GnRH	TAI		

PGF = prostaglandin $F_{2\alpha}$, GnRH = gonadotropin-releasing hormone, TAI = timed artificial insemination.

Taken together, G6G (Bello et al., 2006) and Double-Ovsynch protocols offer new strategies to improve fertility to first timed AI in lactating dairy cows. Both strategies aim to improve fertility by resolving the anovular condition before the timed AI, more tightly controlling follicular development and luteal regression, and initiating Ovsynch on approximately Day 6 or 7 of the estrous cycle when an ovulatory follicle is present and when P4 is increasing during growth of the new follicular wave. Although some dairies have already adopted these protocols for managing first timed AI, more data is needed to make strong recommendations for their use.

Fertility to Timed AI vs. AI after Estrus

The idea that fertility programs and timed AI can yield greater fertility than AI to estrus at first insemination in high-producing dairy cows has not been definitively tested. Several experiments compared P/AI of cows inseminated after timed AI with cows inseminated after a detected estrus at first AI, with some studies reporting no differences in P/AI (Pursley et al., 1997; Chebel & Santos, 2010; Dolecheck et al., 2016) whereas others reported more P/AI for cows receiving timed AI (Gumen et al., 2012; Fricke et al., 2014; Stevenson et al., 2014). In all of these studies, however, DIM at first insemination differed between cows submitted to timed AI and cows submitted to AI after a detected estrus.

We recently conducted an experiment to compare AI submission rate and P/AI between cows submitted to a fertility program versus cows inseminated after an induced estrus (Santos et al., 2017). Lactating Holstein cows were assigned randomly to receive timed AI after a Double-Ovsynch protocol (**DO**) that included a second $PGF_{2\alpha}$ treatment or to receive AI if detected in estrus after receiving 2 $PGF_{2\alpha}$ treatments administered 14 d apart (Estrus). Cows in the Estrus group were induced to cyclicity by treatment with GnRH 7 days before the first $PGF_{2\alpha}$ treatments. Treatments were administered to allow cows to be inseminated at similar DIM in both treatments (77 ± 3 DIM).

Table 4. Effect of treatment on submission rate, pregnancies per AI (P/AI) 33 and 63 d after insemination, and percentage of pregnant cows at 33 and 63 d after insemination in lactating Holstein cows. Adapted from Santos et al., 2017.

	Treatment[1]		Difference,[2]
Item	Double-Ovsynch	Estrus	% (P-Value)
n	294	284	
Submission rate, % (no./no.)	100.0 (294/294)	77.5 (220/284)	29 (<0.01)
P/AI at 33 d, % (no./no.)	49.0 (144/294)	38.6 (85/220)	27 (0.02)
Pregnant cows at 33 d, % (no./no.)	49.0 (144/294)	29.9 (85/284)	64 (<0.01)
P/AI at 66 d, % (no./no.)	44.6 (131/294)	36.4 (80/220)	23 (0.05)
Pregnant cows at 66 d, % (no./no.)	44.6 (131/294)	28.2 (80/284)	58 (<0.01)

[1]Each week, lactating Holstein cows at 50 ± 3 DIM (d 0) were stratified by parity (primiparous vs. multiparous) and were randomized to receive first insemination as a timed AI after a Double-Ovsynch protocol or AI to a detected estrus after a hormonal protocol that synchronized estrus.

[2]Relative difference due to treatment for each item was calculated as the difference between Double-Ovsynch and Estrus cows divided by Estrus cows.

Results from this experiment are summarized in Table 4. By design, DIM at first insemination did not differ between treatments (76.9 ± 0.2 vs. 76.7 ± 0.3 for DO vs. Estrus cows, respectively), but more DO cows were inseminated within 7 d after the end of the voluntary waiting period than Estrus cows (100.0% vs. 77.5%). Overall, DO cows had more P/AI than Estrus cows at both 33 d (49.0% vs. 38.6%) and 63 d (44.6% vs. 36.4%) after insemination, but pregnancy loss from 33 to 63 d after insemination did not differ between treatments. Thus, the relative increase in P/AI was more than 25% (10.4/38.6 = 26.9%). Primiparous cows had more P/AI than multiparous cows 33 and 63 d after insemination, but the treatment by parity interaction was not significant. Synchronization rate to the hormonal protocols was 85.3%, which did not differ between treatments; however, synchronized DO cows had more P/AI 33 d after insemination than synchronized Estrus cows (54.7% vs. 44.5%).

In summary, submission of lactating Holstein cows to a Double-Ovsynch protocol and timed AI for first insemination increased the percentage of cows inseminated within 7 d after the end of the VWP and increased P/AI at 33 and 63 d after first insemination resulting in 64% and 58% more pregnant cows, respectively, than submission of cows for first AI after detection of estrus at a similar day in milk range. We concluded that, because the proportion of synchronized cows did not differ between treatments, DO cows had more P/AI than Estrus cows because of an intrinsic increase in fertility after submission to a fertility program. Thus, use of a higher fertility program like Double-Ovsynch with a second $PGF_{2\alpha}$ treatment can increase service rate and can also increase fertility due to optimization of hormonal concentrations and ovarian dynamics during the protocol.

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