

# **The Implementation and Evolution of Timed Artificial Insemination Protocols for Lactating Dairy Cows**

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## **Introduction**

Reproductive physiologists had long searched to develop a synchronization program that could overcome the problems and limitations associated with visual estrus detection. Such a program was developed at the University of Wisconsin-Madison in 1995 (Pursley et al., 1995) and is now commonly referred to as Ovsynch. Ovsynch synchronizes follicular development, luteal regression and ovulation such that artificial insemination can be conducted at a fixed-time without the need for estrus detection. Subsequent studies that repeated this work soon verified the results of the original publication (Burke et al., 1996; Pursley et al., 1997a,b), and dairy producers soon began to implement the Ovsynch protocol as a tool for reproductive management.

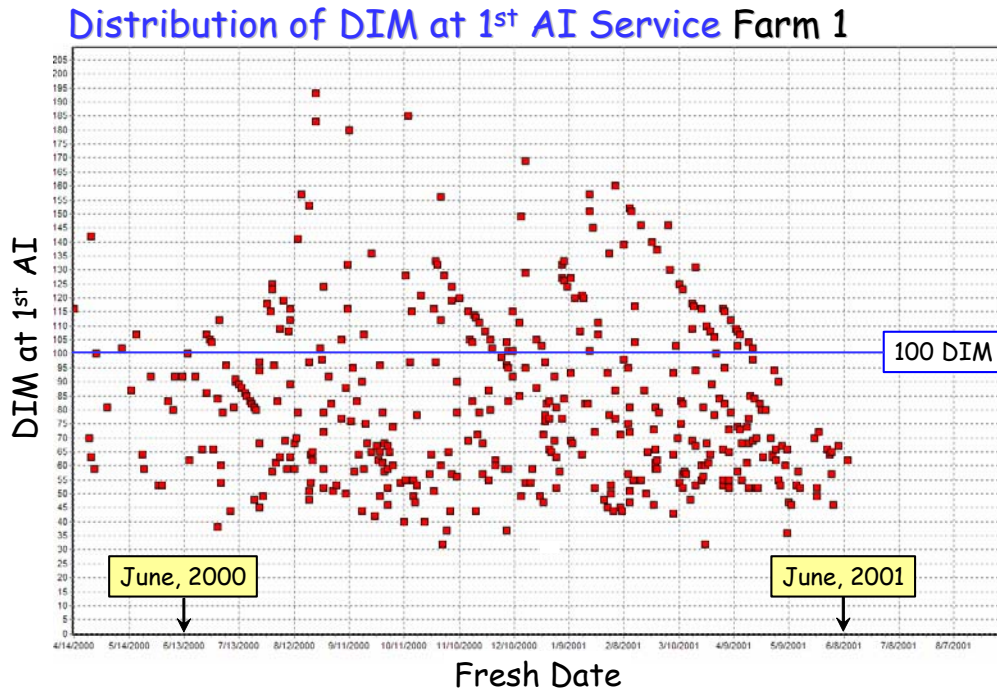
As scientists and dairy producers began to research and implement this new technology, an interesting interplay began to occur between the researchers developing these programs and the dairy managers who had to implement these protocols into their operations. Researchers began to modify the original Ovsynch protocol to try to improve synchrony and fertility to the protocol through presynchronization, altering the timing of AI in relation to ovulation, and testing the various injection intervals of the original protocol. However, reproductive management protocols that allow for TAI and minimize or eliminate visual estrous detection must be practical to implement within the day to day operation of a dairy farm or the protocol will fail due to lack of compliance (Fricke, 2003), and this concept was not first and foremost in the minds of many in the research community. Thus, dairy producers began to modify the published scientific protocols to fit into the day-to-day operation of their reproductive management program which, in turn, initiated more applied research. Although this interplay is ongoing, we are rapidly converging on the few options that blend the best that research has to offer with ease of implementation on a dairy.

This overview begins with the implementation of Ovsynch into a reproductive management system as a treatment for cows failing to be detected in estrus, and ends with a systematic program for synchronization and resynchronization of lactating dairy cows with little or no estrus detection at all. Further research will continue to refine systems for implementation of systematic breeding programs for lactating dairy cows.

## **Incorporating Timed Artificial Insemination into a Reproductive Management System**

It is a fundamental principle of reproductive biology that inseminating a cow is the first step toward establishing a pregnancy. Unfortunately, many cows do not receive their first postpartum AI service until after 100 days in milk. First postpartum AI service represents a unique opportunity for reproductive management of lactating dairy cows because all cows in the herd have a known pregnancy status at this time (e.g., nonpregnant), which allows for use of

hormonal synchronization systems that use  $\text{PGF}_{2\alpha}$  without the risk of aborting a previously established pregnancy. Furthermore, reducing the interval from calving until first AI service for all cows in the herd has a profound effect on reproductive efficiency. The interval that must elapse from calving until a cow is eligible to receive her first AI service is termed the Voluntary Waiting Period (VWP). As the name implies, the duration of this interval is voluntary (i.e., a management decision) and traditionally varies from 40 to 70 days on most dairies.



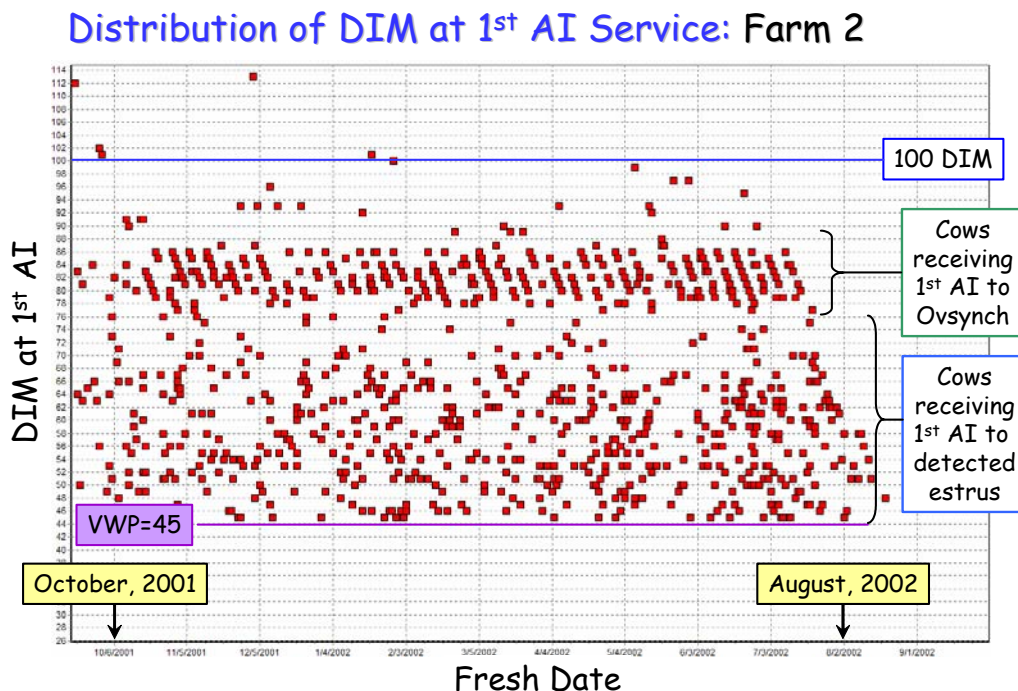
**Figure 1.** Days in milk at first breeding (y-axis) by date of freshening (x-axis) for cows managed using visual detection of estrus for first postpartum AI service. In this herd, nearly one-third of the cows are serviced for the first time after 100 DIM.

Figure 1 illustrates the inefficiency of estrus detection for submitting cows to first AI service for a 500-cow herd managed using visual detection of estrus for first postpartum AI service. Days in milk (DIM) at first breeding is plotted on the vertical axis (y-axis) and date of freshening (i.e., time) is plotted on the horizontal axis (x-axis). Each square represents an observation, or a cow within the herd, and a bold line has been drawn horizontally at 100 DIM. Cows receiving first AI service before 100 DIM fall below the bold line, whereas cows receiving first AI service after 100 DIM fall above the bold line. Nearly one-third of the cows in the herd shown in the upper panel of Figure 1 exceed 100 DIM before first AI service. It should be obvious that none of these cows has a chance of becoming pregnant before 100 DIM because they have not yet been inseminated. Although most dairy producers identify a set duration for the VWP, breeding decisions for individual cows often occur before the VWP elapses. The VWP for the farm illustrated in Figure 1 is 50 DIM; however, many cows are submitted for AI before this time. The decision to AI a cow for the first time postpartum is determined based on when (or if) a cow is detected in estrus rather than on a predetermined management decision. In such instances, the cow is managing the decision to breed rather than the dairy manager. The decision to inseminate a cow before the VWP elapses is motivated by one factor, and that factor is fear. Most producers

fear the decision to not breed a cow detected in estrus because she may not be detected in estrus again until much later in lactation. Unfortunately, this risk is often realized on dairies that rely on visual estrus detection for AI because of poor estrus detection by dairy personnel and poor estrus expression by lactating dairy cows. Recent reports have estimated that 20-30% of lactating cows were not cycling by 60 DIM (Pursley et al., 2001; Gumen et al., 2003). If Figure 1 reflects the reproductive performance to first AI on your farm, you should consider using a controlled breeding program to initiate first postpartum AI service.

### Implementation of Ovsynch using the “Back-Door” Approach

Ovsynch synchronizes follicular development, luteal regression, and time of ovulation, thereby allowing for TAI after the second GnRH injection and improving the AI service rate (Pursley et al., 1995). Ovulation of a dominant follicle in response to the second GnRH injection occurs in around 85% of high producing lactating cows receiving this protocol (Fricke et al., 1998), and ovulation occurs within 24 to 32 h after the second GnRH injection in synchronized cows followed by growth of a new follicular wave (Pursley et al., 1995). Using a 50 µg dose (1.0 ml) of Cystorelin for each injection of the Ovsynch protocol results in similar synchronization and conception rates as using a 100 µg dose (2.0 ml) of Cystorelin (Fricke et al., 1998). Although a reduced dose of Cystorelin has been shown to be effective, the labeled dose of PGF<sub>2α</sub> should be used for all TAI protocols.



**Figure 2.** Days in milk at first breeding (y-axis) by date of freshening (x-axis) for cows managed using a combination of visual detection of estrus and Ovsynch and timed AI for first postpartum AI service. In this herd, cows failing to be detected in estrus during the first 25 days after the voluntary waiting period initiate Ovsynch beginning around 70 days in milk.

Figure 2 illustrates a 1,600-cow herd managed using a combination of visual detection of estrus and Ovsynch and TAI for first postpartum AI service. Similar to Figure 1, DIM at first breeding is plotted on the vertical axis (y-axis) and date of freshening (i.e., time) is plotted on the horizontal axis (x-axis). In this herd, cows failing to be detected in estrus during the first 25 days after the voluntary waiting period (d 45 to 70) initiate the first GnRH injection of Ovsynch beginning around 70 days in milk and receive a TAI 10 d later around 80 DIM. This system is sometimes referred to as the “back-door” Ovsynch approach because Ovsynch is used as a “clean-up” system for cows failing to be detected in estrus. It is not uncommon for Ovsynch to result in a lower conception rate than AI to a detected estrus when analyzing and comparing conception rates in a herd using the back-door Ovsynch approach. This is likely because cows fail to be detected for reasons due to sickness or injury, or because these cows are anovular and not cycling. If this is the case, the expectation should be for a lower conception rate to Ovsynch because a subset of cows with poor fertility is exposed to Ovsynch, whereas normally cycling cows are inseminated to a detected estrus.

Many studies have shown Ovsynch to be a highly effective and economical strategy for improving reproductive performance in high-producing lactating dairy cows (Burke et al., 1996; Pursley et al., 1997a, b; Britt and Gaska, 1998). The first studies comparing use of Ovsynch in which conception rates of lactating dairy cows managed in confinement-based dairies receiving Ovsynch were similar to that of cows receiving AI after a standing estrus (Pursley et al., 1997a,b). However, several subsequent studies have reported that Ovsynch results in lower conception rates compared with AI after estrus (Jobst et al., 2000; Stevenson et al., 1999). In addition, the effectiveness of Ovsynch for breeding lactating dairy cow managed in grazing-based dairies remains equivocal (Cordoba and Fricke, 2001, 2002). Factors explaining the variation in conception rate to TAI among herds are unknown at this time but may include the proportion of anovular cows in the herd, the follicular dynamics of individual cows within the herds, or the ability of farm personnel to implement Ovsynch in their herds.

### **Programming Cows for First Postpartum AI using Presynch/Ovsynch**

The first results with Ovsynch (Pursley et al., 1995) indicated that all nonpregnant cows could be enrolled into the protocol regardless of their stage during the estrous cycle. Subsequent results from Vasconcelos et al. (1999) using lactating dairy cows, and those of Moreira et al. (2000a) using dairy heifers showed that initiation of Ovsynch between days 5 to 12 of the estrous cycle may result in improved conception rate over the original Ovsynch protocol. Hormonal presynchronization of cows to group randomly cycling cows to initiate Ovsynch between days 5 to 12 of the estrous cycle can be accomplished using two injections of PGF<sub>2α</sub> administered 14 days apart before initiation of the first GnRH injection of Ovsynch. A presynchronization strategy in which two injections of PGF<sub>2α</sub> administered 14 d apart preceded initiation of Ovsynch by 12 d has shown to improve conception rate in lactating dairy cows compared to Ovsynch (Moreira et al., 2000c). Lactating dairy cows were randomly assigned to receive Ovsynch (n=262) or Presynch (n=264) for their first postpartum TAI, which was conducted 16 h after the second GnRH injection. The first and second PGF<sub>2α</sub> injections for Presynch cows were administered at 37 and 51 days in milk, respectively, and all cows received a TAI at 73 days in milk. One possible hormone injection and timed AI schedule based on this research is shown in Table 1. For cycling cows, conception rate increased from 29% for Ovsynch to 43% for Presynch cows; however, no statistical treatment difference was detected when all cows (cycling

and anovular) were included in the analysis). Thus, use of Presynch for programming lactating dairy cows to receive their first postpartum TAI can improve first service conception rate in a dairy herd.

**Table 1.** One possible hormone injection and timed artificial insemination schedule for the Presynch/Ovsynch protocol based on the results of Moreira et al., 2000c.

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.

A common question regarding the original Presynch data from Moreira et al. (2000c) pertains to the importance of the 12-day interval between the second PGF<sub>2α</sub> injection and the first GnRH injection. If this interval could be extended to 14 rather than 12 days, the first four injections could be scheduled to occur on the same day during successive weeks. This becomes important for compliance on dairy farms that assign groups of cows to initiate the protocol weekly so that injection schedules do not get confused among the groups. To determine if two injections of PGF<sub>2α</sub> 14 d apart administered 14 d before initiation of Ovsynch, would change follicular dynamics, ovulation rate, and conception rate in lactating dairy cows (Navanukraw et al., 2004), nonpregnant lactating Holstein cows (n=257) >60 DIM were blocked by parity and were randomly assigned to each of two groups. Cows in the first group (Ovsynch, n=128) received 50 µg GnRH (d -10); 25 mg PGF<sub>2α</sub> (d -3) and 50 µg GnRH (d -1) beginning at a random stage of the estrous cycle. Cows in the second group (Presynch, n=129) received Ovsynch but with the addition of PGF<sub>2α</sub> (25 mg) injections on d -38 and -24. All cows received TAI (d 0) 18 h after the second GnRH injection. One possible hormone injection and timed AI schedule based on this research is shown in Table 2. The only advantage of this modified protocol is that the first four injections of the protocol can be scheduled for the same day of the week during successive weeks. This becomes important for compliance by dairy producers that assign groups of cows to initiate the protocol on a weekly basis so that administration schedules do not get confused among groups of cows (Fricke et al., 2003).

**Table 2.** One possible hormone injection and timed artificial insemination schedule for the Presynch/Ovsynch protocol based on the results of Navanukraw et al., 2004.

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.

As shown in Table 3, although the proportion of cows ovulating after the first and second GnRH injections did not differ statistically between treatments, conception rate was greater ( $P<0.05$ ) for cows receiving Presynch vs. Ovsynch. These data support use of this modified Presynch protocol

to increase conception rate of lactating dairy cows receiving TAI, and many dairies using Presynch have incorporated this modified protocol. In agreement with our hypothesis, PR/AI of cows receiving the modified Presynch protocol was greater ( $P < 0.05$ ) than that of cows receiving Ovsynch (Table 3). This observation supports and extends previous reports in which presynchronization using two injections of PGF<sub>2α</sub> 14 d apart beginning 26 d before initiation of Ovsynch increased PR/AI to TAI in lactating dairy cows compared with Ovsynch (Moreira et al., 2001; El-Zarkouny et al., 2002).

**Table 3.** Proportion of cows ovulating after the first and second GnRH injections and pregnancy rate per artificial insemination (PR/AI) to TAI for lactating dairy cows receiving Ovsynch or a modified Presynch protocol (Adapted from Navanukraw et al., 2004).

Item	Treatment <sup>1</sup>	
	Ovsynch	Presynch
Synchronized ovulation rate		
Ovulation after first GnRH (%) (no./no.)	41.1 (23/56)	35.9 (19/53)
Ovulation after second GnRH (%) (no./no.)	69.6 (39/56)	81.1 (43/53)
PR/AI (%) (no./no.)	37.3 <sup>a</sup> (50/134)	49.6 <sup>b</sup> (67/135)

<sup>a,b</sup>Proportions differ ( $P < 0.05$ ).

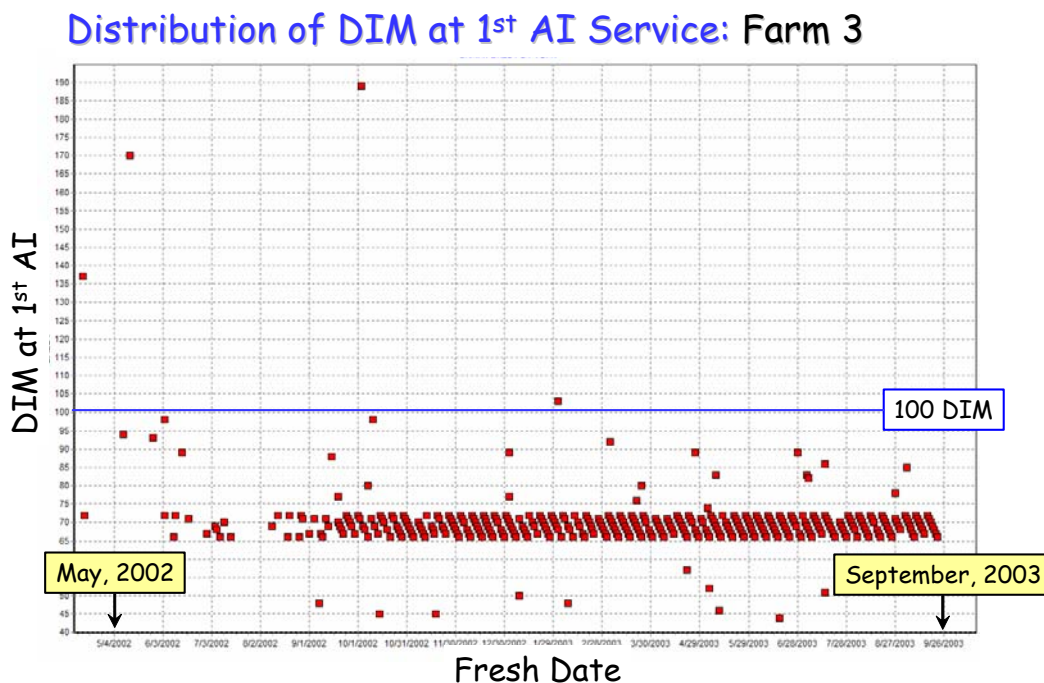
#### *How Might Presynch Improve Fertility?*

Although several studies have reported PR/AI increases due to Presynch using two injections of PGF<sub>2α</sub> (Moreira et al., 2001; El-Zarkouny et al., 2002; Navanukraw et al., 2004), specific mechanisms by which presynchronization improves PR/AI remain to be determined. One possible mechanism by which Presynch may improve PR/AI is by presynchronizing the estrous cycle so that cows initiate the first injection of Ovsynch at a specific stage of the cycle. Initiation of Ovsynch on d 5 to 9 of the estrous cycle in lactating dairy cows resulted in a greater synchronization rate and PR/AI compared to other stages of the cycle (Vasconcelos et al., 1999). Administration of the first GnRH injection of Ovsynch on d 5 to 10 of the estrous cycle may increase the probability of ovulating the dominant follicle of the first follicular wave of the estrous cycle, thereby improving synchrony of emergence of a new wave and synchronized ovulation rate to the second GnRH injection of Ovsynch. The stage of the cycle at administration of the first GnRH injection of Ovsynch on d 5 to 10 of the estrous cycle may provide a more favorable P<sub>4</sub> environment during development of the ovulatory follicle which may affect PR/AI.

Data supporting a positive effect of high P<sub>4</sub> at initiation of Ovsynch on PR/AI are equivocal. A single injection of PGF<sub>2α</sub> administered 10 d before initiation of Ovsynch to lactating cows in five commercial herds in Ontario, Canada did not improve conception rate compared to Ovsynch alone (LeBlanc and Leslie, 2003). Administration of PGF<sub>2α</sub> to lactating cows 12 d before the first GnRH injection of Ovsynch shifted cows into the early luteal phase of the estrous cycle at the time of the first GnRH injection, but no improvement in reproductive performance was observed compared with Ovsynch (Cordoba and Fricke, 2001). By contrast, a single injection of PGF<sub>2α</sub> administered 12 d before Ovsynch shifted cows to early diestrus at the first GnRH injection but increased PR/AI only in multiparous but not primiparous cows compared with Ovsynch

(Cartmill et al., 2001). When lactating cows were presynchronized with  $\text{PGF}_{2\alpha}$  10 d and GnRH 7 d before initiation of Ovsynch, the increase in the proportion of luteal-phase cows at initiation of Ovsynch did not affect PR/AI to TAI (Peters and Pursley, 2002).

One possible explanation for the increase in PR/AI using two injections of  $\text{PGF}_{2\alpha}$  compared with other presynchronization strategies is that two successive injections of  $\text{PGF}_{2\alpha}$  may exert a positive effect on the uterine environment. In lactating dairy cows, PR/AI increased with increasing number of estruses occurring during the postpartum period (Thatcher and Wilcox, 1972), and a positive effect of  $\text{PGF}_{2\alpha}$  administration on PR/AI has been reported in cattle (Roche, 1976; Macmillan and Day, 1982). Cycling cows should express estrus once if not twice before TAI when subjected to a Presynch protocol using two injections of  $\text{PGF}_{2\alpha}$  administered 12 or 14 d apart. In support of this idea, cows that were cycling at initiation of presynchronization using two injections of  $\text{PGF}_{2\alpha}$  exhibited increased PR/AI, but when anovular cows were included in the analysis, no effect of presynchronization was detected (Moreira et al., 2001). Exogenous  $\text{PGF}_{2\alpha}$  may also act directly through upregulation of a uterine immune response as demonstrated in sows (Wulster-Radcliffe et al., 2001) and ewes (Lewis and Wulster-Radcliffe, 2001). Further research is needed to fully understand the mechanisms by which presynchronization increases PR/AI in lactating dairy cows.



**Figure 3.** Days in milk at first breeding (y-axis) by date of freshening (x-axis) for cows managed using Presynch/Ovsynch and timed AI for first postpartum AI service. In this herd, 98% of cows receive a timed AI for first service and less than 5% of cows receive AI after a detected estrus.

### Implementation of a Presynch/Ovsynch Protocol

Use of a controlled breeding program such as Presynch/Ovsynch for initiating first AI service exposes all cows in the herd to the risk of becoming pregnant at or very near the end of the VWP. Figure 3, illustrates a 1,100-cow herd managed using the Presynch/Ovsynch schedule shown in Table 2. Similar to Figures 1 and 2, DIM at first breeding is plotted on the vertical axis (y-axis) and date of freshening (i.e., time) is plotted on the horizontal axis (x-axis). In this herd, nearly all cows receive their first postpartum AI service between 65 and 73 DIM. In this scenario, the end of the VWP is roughly equal to the average day at first service for the entire herd. Of course, not all cows will conceive to first service; conception rates in lactating dairy cows are poor, and hormonal breeding programs increase pregnancy rate by increasing service rate, not conception rate.

### When to Conduct Timed AI after the Second GnRH Injection

Reproductive management protocols that allow for TAI and minimize or eliminate visual estrous detection must be practical to implement within the day to day operation of a dairy farm or the protocol will fail due to lack of compliance (Fricke, 2003). It is common for farms to choose to adopt a TAI schedule that represents a variation in which the TAI is performed during the same cow-handling period as the second GnRH (i.e., Cosynch), thereby eliminating one handling period compared with the first reported Ovsynch protocol (Pursley et al., 1995). Although the timing of insemination in a Cosynch strategy may not maximize conception rate to TAI (Pursley et al, 1998; Dalton et al., 2001), use of Cosynch allows for cows to be handled at the same time of the day on different days, thereby allowing for cows to be restrained in self-locking head gates or a palpation rail after a specified milking in 3X milking systems in which cow-handling periods are dictated by the milking routine rather than by pre-selected protocol intervals.

To assess the optimal time of AI in relation to synchronized ovulation, lactating dairy cows (n = 733) from Wisconsin dairy herds with 22,000 to 26,000 pound rolling herd averages were randomly assigned to five groups by stage of lactation and parity (Pursley et al., 1998). Ovulation was synchronized using Ovsynch, and cows received AI at 0, 8, 16, 24, or 32 hours after the second injection of GnRH. In this study, the 0 h group is equivalent to the Cosynch protocol. As determined in a preliminary study, all cows ovulate 24 to 32 hours after the second GnRH injection. Injection times were varied so that all cows were inseminated at the same time, and the inseminators were blind to treatment group. Pregnancy status was determined 25 to 35 days after AI for all groups by using transrectal ultrasonography.

**Table 4.** Reproductive measures in lactating dairy cows inseminated at various times in relation to ovulation synchronized with an injection of GnRH (Adapted from Pursley et al., 1998). In this experiment, the 0 hour group is equivalent to Cosynch.

Item	Hours from second GnRH injection to TAI					Total
	0	8	16	24	32	
n	149	148	149	143	143	732
Conception rate (%)	37	41	45	41	32**	39
Pregnancy loss (%)	9**	21	21	21	32	22
Calving rate (%)	31	31	33	29	20*	29

\*Differs within a row ( $P<0.05$ ).

\*\*Differs within a row ( $P<0.10$ ).

Conception rate and calving rate was greater ( $P<0.05$ ) for cows in the 0, 8, 16, and 24-hour groups compared with the 32-hour group (Table 4). Pregnancy loss was less ( $P<0.05$ ) for the 0 hour group compared with all other groups, and there was a tendency for greater pregnancy loss in the 32-hour group ( $P<0.1$ ; Table 4). Thus, although no statistical difference in conception rate occurs when breeding from 0 to 24 hours after the second GnRH injection, breeding too late (i.e., at 32 hours) decreases conception rate.

### A Comparison of Combinations of Presynch/Ovsynch and Presynch/Cosynch

A recent column in Hoard's Dairyman (Stevenson, 2004), presented unpublished results from a field trial conducted on two dairies in northeastern Kansas to compare various combinations of Presynch/Ovsynch and Presynch/Cosynch. All cows were <40 DIM at the initiation of the Presynch protocol and they were milked three times daily and produced in excess of 10,000 kg milk. All cows received two 25-mg injections of PGF<sub>2α</sub> 14 days apart, with the second injection administered 12 days before initiating the first GnRH injection of Ovsynch. Cows were blocked by lactation number and randomly assigned to three treatments (A, B, and C). Cows in treatments A and B received two injections of GnRH 7 d before and 48 h after PGF<sub>2α</sub>. Timed AI was conducted at the time of the second GnRH injection (i.e., Cosynch; treatment A) or 24 h later (treatment B). Cows in treatment C received the second GnRH injection 72 h after PGF<sub>2α</sub> and received TAI at the same time. All cows in each of the three treatments were inseminated via TAI regardless of whether they displayed estrus before the scheduled time of TAI. Results are shown in Table 5.

**Table 5.** Reproductive measures in lactating dairy cows inseminated at various times in relation to ovulation synchronized with an injection of GnRH (Adapted from Stevenson, 2004).

Herd	Treatment			Overall
	A	B	C	
1	16 (80)	19 (78)	24 (76)	20 <sup>a</sup> (234)
2	27 (141)	34 (147)	41 (144)	34 <sup>b</sup> (432)
Overall	23 <sup>c</sup> (221)	29 <sup>c</sup> (225)	35 <sup>d</sup> (220)	29 (666)

<sup>a,b</sup>Herd effect ( $P<0.01$ ).

<sup>c,d</sup>Treatment effect ( $P<0.05$ ).

In this study, TAI at 0 and 24 h after GnRH when GnRH was administered 48 h after PGF<sub>2α</sub> resulted in lower fertility than conducting the GnRH and TAI at 72 h after PGF<sub>2α</sub>. Treatment C allows time and labor savings because cows receive TAI and injections on the same day and all treatments and TAI can occur at the same time of the day (i.e., all in the AM or all in the PM). One possible hormone injection and timed AI schedule based on treatment C from this research is shown in Table 6.

**Table 6.** One possible hormone injection and timed artificial insemination schedule for the Presynch/Ovsynch protocol based on the results of Navanukraw et al., 2004.

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

PGF = prostaglandin F<sub>2α</sub>, GnRH = gonadotropin-releasing hormone, TAI = timed artificial insemination.

## **Programming First and Second AI Service: Presynch and Resynch**

Aggressive reproductive management comprises three strategies that can be implemented early during the breeding period of lactating dairy cows: 1) submit all cows for first postpartum AI service at the end of the voluntary waiting period, 2) identify nonpregnant cows post-AI, and 3) return cows failing to conceive to first AI service to second AI service. Timely rebreeding of lactating dairy cows that fail to conceive to first AI service is essential for improving reproductive efficiency and profitability in a dairy herd. Because AI conception rates of high producing lactating dairy cows are reported to be 40% or less (Pursley et al., 1997a; Fricke et al., 1998), 60% or more of lactating cows will fail to conceive to a given AI service. Now that it is relatively easy to program cows for first postpartum AI service, many producers are asking how best to identify nonpregnant cows and program them for second and subsequent AI services.

We recently conducted a field trial to test such a system on a dairy (Fricke et al., 2003). Our objective was to compare conception rate to first TAI service after a modified Presynch protocol with conception rates after resynchronization of ovulation using Ovsynch at three intervals post TAI (Resynch). Lactating dairy cows (n =711) on a commercial dairy farm in North-central Wisconsin were enrolled into this study on a weekly basis beginning on May 10, 2001 and ending on May 30, 2002. All cows received a modified Presynch protocol to receive first postpartum TAI as follows: 25 mg PGF<sub>2α</sub> (d 32 ± 3; d 46 ± 3); 50 µg GnRH (d 60 ± 3); 25 mg PGF<sub>2α</sub> (d 67 ± 3) and 50 µg GnRH (d 69 ± 3) postpartum. All cows received TAI immediately after the second GnRH injection of the Presynch protocol (d 0) as per a Cosynch TAI schedule. At first TAI, cows were randomly assigned to each of three treatment groups for resynchronization of ovulation (Resynch) using Ovsynch [50 µg GnRH (d -9); 25 mg PGF<sub>2α</sub> (d -2) and 50 µg GnRH + TAI (d -0)] to induce a second TAI for cows failing to conceive to first TAI service. All cows (n=235) in the first group (Day 19) received a GnRH injection on d 19 post TAI and continued the Ovsynch protocol if diagnosed nonpregnant using transrectal ultrasound on d 26 post TAI. Cows (n=240) in the second (Day 26) and cows (n=236) in the third (Day 33) groups initiated the Ovsynch protocol if diagnosed nonpregnant using transrectal ultrasound on d 26 post-TAI or d 33 post-TAI, respectively.

Implicit to the experimental design, first assessment of pregnancy status was not conducted at the same interval after the Ovsynch TAI among the three treatment groups. Pregnancy status after the Ovsynch TAI was first assessed 26 d after TAI for cows in the D19 and D26 groups, whereas pregnancy status was assessed 33 d post Ovsynch TAI for cows in the D33 group (Table 7). Overall PR/AI to Ovsynch was 40 % (286/711) and was greater for D19 and D26 cows than for D33 cows (Table 7). This difference is likely due to a greater period in which embryonic loss 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 treatment groups at 68 d after Ovsynch TAI, overall PR/AI to Ovsynch was 31 % (219/711) and did not differ among treatments (Table 7). Thus, differences in PR/AI at the first pregnancy exam and pregnancy losses between the first and second pregnancy exams among treatment groups likely represent an artifact of time of assessment of pregnancy status after TAI inherent to the experimental design rather than to treatment differences.

**Table 7.** Pregnancy rate per artificial insemination (PR/AI) and pregnancy loss after timed artificial insemination (TAI) to Ovsynch (Adapted from Fricke et al., 2003).

Item	Treatment group			Overall
	D19	D26	D33	
Interval from Ovsynch TAI to 1 <sup>st</sup> pregnancy exam (d)	26	26	33	-
PR/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	-
PR/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 treatment groups.

A total of 41 cows diagnosed nonpregnant to Ovsynch were not enrolled for Resynch for second TAI service for various reasons. Only 5.6 % of cows failing to conceive to Ovsynch were visually detected in estrus and inseminated, underscoring the reliance of this farm on TAI for inseminating cows. Overall PR/AI to Resynch was 32 % and was greater for D26 and D33 cows than for D19 cows (Table 6). Although PR/AI to TAI protocols can vary widely among farms, PR/AI after Resynch for the D26 and D33 cows in the present study is similar to conception rate after Ovsynch reported previously (Pursley et al., 1995; Fricke et al., 1998; Jobst et al., 2000).

**Table 8.** Pregnancy rate per artificial insemination (PR/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 group			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)	-
PR/AI, % (no./no.)	23 <sup>a</sup> (28/120)	34 <sup>b</sup> (41/121)	38 <sup>b</sup> (54/143)	32 (123/384)

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

Specific reasons for the poor Resynch PR/AI for D19 cows in the present study are unknown. Success of the Ovsynch protocol in lactating dairy cows varies depending on cyclicity status and stage of the estrous cycle at initiation of Ovsynch (Moreira et al., 2001). Stage of the estrous cycle at initiation of Ovsynch affects synchronized ovulation rate to the first GnRH injection and subsequent conception rate to TAI (Vasconcelos et al., 1999; Moreira et al., 2000a). In addition, cows initiating the Ovsynch protocol during early to mid diestrus (d 5 to 12 of the estrous cycle) when serum progesterone concentrations were high, ovulated smaller follicles and had greater conception rates than cows initiating Ovsynch during metestrus, late diestrus, or proestrus (Vasconcelos et al., 1999). Assuming an average estrous cycle duration of 23 d (Savio et al., 1990; Pursley et al., 1993; Sartori, 2002) for cows failing to conceive to Ovsynch, initiation of Resynch in the present study would approximately coincide with three stages of the estrous

cycle: proestrus (D19), metestrus (D26), and diestrus (D33). Under this scenario and based on previous reports (Vasconcelos et al., 1999; Moreira et al., 2000a), initiation of Resynch 33 d after TAI should yield the highest PR/AI. In the present study, PR/AI to Resynch was numerically greatest (38%) for the D33 group but not statistically greater than the D26 group (Table 8). This occurred in spite of the longer interval from Resynch TAI to pregnancy diagnosis in the D33 group (33.7 d) compared to the D26 group (26.6 d) and in addition to the expected increased incidence of embryonic loss among treatment groups similar to that observed for PR/AI to Ovsynch.

Results from this study argue against the D19 group as a viable resynchronization strategy based on the poor PR/AI after the Resynch TAI. A veterinarian who can accurately determine pregnancy status via rectal palpation 33 d post TAI could incorporate the D33 Resynch strategy without reliance on transrectal ultrasound for early pregnancy diagnosis. Assuming that administration of GnRH to pregnant cows 33 d after TAI does not induce iatrogenic embryonic loss, all cows could be administered GnRH at 33 d after TAI. Cows would then receive PGF<sub>2α</sub> at a nonpregnancy diagnosis via rectal palpation conducted one week later. One possible hormone injection and timed AI schedule based on this research is shown in Table 9.

**Table 9.** One possible hormone injection and timed artificial insemination schedule for the Presynch/Ovsynch protocol for first TAI and Resynchronization for second TAI based on the results of Fricke et al., 2003.

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

PGF = prostaglandin F<sub>2α</sub>, GnRH = gonadotropin-releasing hormone, TAI = timed artificial insemination, PG = pregnancy diagnosis

It is possible that the optimal resynchronization strategy for this herd may not perform optimally in other herds. A difference in populations with varying proportions of cows exhibiting two or three follicular waves per cycle has been suggested to impact conception rate to Ovsynch (Cordoba and Fricke, 2002). Further research is needed to further develop successful resynchronization strategies for managing reproduction in lactating dairy cows.

### Embryonic Loss and Pregnancy Rechecks

Pregnancy loss contributes to reproductive inefficiency because fertility assessed at any point during pregnancy is a function of both conception rate and pregnancy loss (Fricke, 2002). For cows diagnosed pregnant to Ovsynch, overall pregnancy loss occurring between the first pregnancy exam and 68 d of gestation was 23 % (67/286) and was greater for D19 and D26 cows

than for D33 cows (Table 7). Although not a direct comparison, the numbers of pregnancies lost in each treatment group almost fully accounted for differences in PR/AI to the first pregnancy exam after the Ovsynch TAI among treatment groups (Table 7). Of cows diagnosed pregnant at 28 d post TAI, 10 to 16 % experience embryonic loss by 56 d after TAI (Mee et al., 1994; Vasconcelos et al., 1997; Fricke et al., 1998). Although the magnitude of embryonic loss in this study is greater than that reported in previous studies, the period over which loss was assessed beginning earlier in gestation (26 to 33 d). In a previous study (Vasconcelos et al., 1997), pregnancy loss in lactating dairy cows was 11% from 28 to 42 d, 6% from 42 to 56 d, and 2% from 56 to 98 d post AI, suggesting that losses are highest early and subsequently decrease as gestation ensues.

Because cows diagnosed pregnant at an early ultrasound exam have a greater risk of early embryonic loss, these cows must undergo subsequent pregnancy examinations to identify and rebreed cows that experience such loss (Fricke, 2002). If left unidentified, cows experiencing embryonic loss after an early pregnancy diagnosis would actually reduce reproductive efficiency by extending intervals from calving to conception. Incorporation of a pregnancy recheck conducted around 61 or 68 d in gestation into a systematic synch and resynch program is essential for identifying cows that experience embryonic loss.

### Protocols that Just Might Work but Really Should be Empirically Tested First

Taken collectively from the data presented thus far, two possible hormone injection and TAI schedules that may work well on farms are shown in Tables 10 and 11. Both schemes incorporate elements tested in previous research but not fully tested as a single scheme in a field trial. Both schemes include a Presynch/Ovsynch strategy for first TAI service (Moreira et al., 2000c; Navanukraw et al., 2004), a 72 h interval from the PGF<sub>2α</sub> injection to the second GnRH and TAI for first AI (Stevenson, 2004) and for resynchronization, and a 32-day interval from first TAI to initiation of Resynch (Fricke et al., 2003). Further research is needed to assess the various untested aspects of these protocols on production dairy farms.

**Table 10.** One possible hormone injection and timed artificial insemination schedule for the Presynch/Ovsynch protocol for first TAI and Resynchronization for second TAI based on the results of Fricke et al., 2003.

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

PGF = prostaglandin F<sub>2α</sub>, GnRH = gonadotropin-releasing hormone, TAI = timed artificial insemination, PG = pregnancy diagnosis

**Table 11.** One possible hormone injection and timed artificial insemination schedule for the Presynch/Ovsynch protocol for first TAI and Resynchronization for second TAI based on the results of Fricke et al., 2003.

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

PGF = prostaglandin F<sub>2α</sub>, GnRH = gonadotropin-releasing hormone, TAI = timed artificial insemination, PG = pregnancy diagnosis

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