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## The Why's, What's, and How's of Timed Artificial Insemination Programs

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### Summary

This report summarizes the benefits of using timed artificial insemination (AI) programs in lactating dairy cows. Since its beginnings, use of Ovsynch has penetrated the dairy industry and is most prevalent in the Midwest and Northeast regions of the U.S. The timed AI program benefits farms that have difficulties with heat detection. Heat detection challenges can occur because of inadequate observation or poor expression of heat associated with footing conditions. Explanation of mechanisms associated with hormone injections are presented to justify the success of currently recommended timed AI programs.

Key words: timed AI, mechanisms, Ovsynch

### Introduction

The successful timed AI method as we know it today was first applied to dairy cows in Wisconsin more than two decades ago, when Ovsynch was introduced by Pursley and Wiltbank. Before that time, inseminations made at 72 to 80 hours after prostaglandin  $F_{2\alpha}$  (PGF) administration did not produce conception rates such as those observed today. Application of Ovsynch has markedly changed how we manage reproduction not only in dairy cows, but also in beef cows. Because of the options provided by timed AI, and where heat detection is lacking, many dairies can manage quite well their AI-breeding program without heat detection. This review will provide an overview of the use of timed AI in the U.S. and some of the characteristics of farms applying estrus- and ovulation-synchronization and timed AI programs, as well as the mechanisms by which fertility can be maximized in timed AI programs.

### Prevalence and Distribution of Timed AI

A recent study consisting of more than 1.1 million breeding records from 40 states was conducted at the University of Wisconsin. The study utilized Dairy Herd Improvement (DHI) records originating from dairy farms that use AGSource, Agritech, and DRMS as their dairy record processing centers. The final data set included AI records from 2008 through 2012, and were restricted to AI breedings with confirmed pregnancy outcomes from herds reporting at least 30 breedings during the preceding 12 months. Breeding codes for each AI were classified within herd as either AI after heats or synchronized AI (timed AI) based on weekly profiles. Farms were assumed

to be using synchronization and timed AI when at least 30% of the inseminations occurred on the same day of the week.

Overall, nearly 30% of all AI breedings occurred as timed AI. Figure 1 shows a large variation in timed AI use across the U.S. The greatest use of timed AI was in the Midwestern and Northeastern states. The leading states in terms of proportion of timed AI use (reporting more than 5,000 breedings) were SD = 56%, WI = 46%, IA = 43%, OH = 40%, MI = 32%, NY = 29%, PA = 27%, MN = 24%, VA = 23%, and SC = 23%. Although more than 78% of timed AI occurred on Thursdays and Fridays, actual conception results did not differ between timed AI (32.6%) and AI after estrus (33.4%; Figure 2). Only during June and July were conceptions after timed AI significantly less than those of cows inseminated after heat detection.

### Why Timed AI?

Employing timed AI overcomes the obstacle of inadequate heat detection, poor expression of estrus, or both. Inadequate heat detection is a people problem and poor expression of heat is a cow or facility problem. Cows housed in dry lots have the advantages of good footing, cow comfort, and heat-detection rates that often exceed 70%. Cows housed entirely on concrete face the challenges of overcrowding, sometimes slippery footing conditions, and inadequate space to demonstrate normal sexual behavior. As a result, heat-detection rates are often more average (50%) for cows housed in total confinement with only concrete surfaces.

Timed AI is often employed in several ways in different herds. In herds with superior heat-detection rates, timed AI is the catch-all for cows that fail to be observed in estrus. In contrast, herds with poor heat detection tend to use more timed AI and employ some cherry-picking of cows detected in heat.

### How Does Ovsynch Work?

To better understand how an Ovsynch program (Figure 3) works, we will consider the different types of cows that begin the program. This scenario provides that no presynchronization has occurred and cows are at random stages of their estrous cycles to start the program. Three types of cows start the program at G1 and ovulation response to G1 varies accordingly: (1) cycling cows with a corpus luteum (CL)—these cows may ovulate to form a second, new CL; (2) cycling cows with no CL—these cows may ovulate to form a new CL; and (3) noncycling cows that have not begun their estrous cycles since calving—these cows may ovulate to form their first post-calving CL, thus initiating their first postpartum estrous cycle.

Ovulation response to G1 depends on two factors: (1) presence of a dominant follicle capable of ovulation; and (2) concentration of progesterone. Progesterone inhibits the amount of ovulation hormone (luteinizing hormone [LH]) released in response to G1. Cows with a CL and larger concentrations of progesterone are less likely to ovulate than cows with CL but with reduced concentrations of progesterone. Therefore, three outcomes are possible 5 or 7 days later (depending on use of the 5- or 7-day Ovsynch program) when PGF is injected. Cows will have: (1) a new CL; (2) an original (pre-G1) CL (original CL); or (3) an original (pre-G1) CL plus a new CL (original + new CL).

When ovulation to G1 occurs, a new growth wave of follicles is initiated approximately 36 to 48 hours after G1 so at the time of PGF injection, a new dominant follicle will exist that is approximately 3 or 5 days old (depending on use of the 5- or 7-day Ovsynch program). When ovulation after G1 does not occur, the dominant follicle is less synchronous with the upcoming PGF injection and subsequent luteal regression (death of the CL).

## Limitations

### *CL Regression*

We expect pregnancy outcomes to be less when: (1) luteal regression is incomplete or does not occur; and (or) (2) the dominant follicle is not responsive to G2 as evidenced by timely ovulation and shedding of the egg into the oviduct. As a result of an ovulation after G1, the age of the CL(s) can be quite variable and alter responsiveness to PGF. Note in Table 1 that regression of the original CL exceeds 95%, but the new CL regression is much less successful at 64%. In contrast, when a new CL and the original CL co-exist, regression success approaches that of the original CL, thus explaining, in part, why fertility is always improved in cows that ovulate in response to G1. The greatest limitation to successful CL regression is when a new CL exists by itself as the only luteal structure at the time of PGF injection.

Research to test the value of including a second PGF injection to maximize CL regression has had mixed success. Increasing the PGF dose by 50% increased CL regression, but did not translate into greater pregnancy outcomes, except in one study in which a second dose of PGF administered 24 hours after the first PGF injection was beneficial in older cows (second lactation and greater).

Results of a recent study are instructive as to the subgroup of cows that benefit from an additional exposure to PGF. Cows were treated with either one or two PGF injections; the first was 7 days after G1 and the second 24 hours after the first PGF injection (Table 2). Cows with low progesterone at G1 (those without a CL) had greater CL regression after two PGF injections. Why? This subgroup of cows are those with the greatest ovulatory response to G1 and would have a new CL, less responsive to PGF as shown in Table 1. Cows with high progesterone at G1 are less likely to ovulate in response to G1 because very high progesterone concentrations inhibit the gonadotropin-releasing hormone (GnRH)-induced LH release necessary to cause ovulation to G1. This subgroup includes cows with at least one original CL before G1 and either none or one new CL after G1. Based on results in Table 1, we would expect CL regression for high progesterone cows to exceed 90%, as evidenced in Table 2 for cows receiving two PGF injections. Pregnancy outcomes at day 32 after timed AI, however, only increased in the low progesterone subgroup when treated with two PGF injections, but also tended to improve in the high progesterone group, thus causing an overall increase in pregnancy outcome for cows receiving two PGF injections (Table 2).

To consider the cows enrolled in the shorter (5-day) Ovsynch option, a recent study compared pregnancy outcomes in cows treated with either the 5- or 7-day timed AI program. All 5-day cows received two PGF injections (5D2PGF), whereas half of the 7-day cows received one PGF injection (7D1PGF), and half received two injections (7D2PGF). Pregnancy outcomes did not differ between the 5- and 7-day programs. In

contrast, when cows received two PGF injections, pregnancy outcome was improved compared with cows receiving but one PGF injection. As shown previously, for cows lacking a CL at G1 and receiving two PGF injections (large proportion of cows with a new CL), pregnancy outcome was greater than when they received but one PGF injection (Table 3). Although it seems that a trend occurred for the same response in cows with a CL at G1, pregnancy outcomes for this group did not differ between cows receiving one or two PGF injections.

### ***Ovulation***

Ovulation response to G2 will occur nearly 100% of the time if luteal regression occurs successfully or progesterone concentrations are very low at the timed AI. Timing of the G2 injection is another critical component to maximizing pregnancy outcome. On the basis of four combined studies, cows to be inseminated at first service and administered G2 at approximately 56 hours after PGF and inseminated approximately 16 hours later (72 hours after PGF) tended to have the best conception rates (Table 4). A similar observation is reported for cows at repeat services.

Why is it better, when practical, to administer G2 approximately 16 hours before insemination? That sequence best mimics what occurs at spontaneous estrus. When a cow starts into heat and stands for the first time in response to a mounting herd mate, two significant hormonal events occur. Heat is caused by estradiol (estrogen) secretion from a mature, preovulatory follicle. Blood concentrations of estradiol peak at the onset of standing heat. Therefore, the peak of estradiol is the trigger to initiate the surge release of GnRH from the hypothalamus in the brain that results in the release (surge) of the LH from the pituitary gland. Concentrations of estradiol and LH peak at or near the onset of standing heat. We expect ovulation of the preovulatory follicle to occur from 24 to 30 hours after the onset of heat, peak in estradiol, and the LH surge. Ideally, according to the a.m.-p.m. rule, we inseminate cows approximately 12 hours after the onset of heat.

In timed AI programs, the trigger to initiate ovulation is G2. Surge release of LH peaks approximately 1 hour after G2 and we expect ovulation to occur 24 to 30 hours later. Therefore, injecting G2 before insemination mimics the natural events of spontaneous estrus and shortens the time to ovulation relative to semen placement. Sperm transport from the uterine body or horns to the utero-tubal junction requires up to 10 hours. Sperm concentrate and await ovulation at the utero-tubal junction. Administering G2 approximately 16 hours before AI allows ovulation to occur approximately 8 to 14 hours after sperm have formed a reservoir at the utero-tubal junction. This timing matches nicely what occurs naturally when cows come into heat spontaneously and ovulate in response to their own estradiol-GnRH-LH signals.

### **Presynchronization Before First Services**

Presynchronizing estrous cycles before the first service can be accomplished with PGF or combinations of GnRH and PGF. Illustrated in Figure 4 are four presynch programs. The PGF-Presynch program is a 14 day – 10 day program (14 days between injections and 10 days between the last PGF-Presynch injection and G1). It is the best PGF-Presynch system because all cows that respond to the second PGF-Presynch injection and expressing estrus between 2 and 5 days after PGF will be on days 5 through 8 of the new

estrous cycle and highly responsive to G1. Remember, better fertility occurs after the timed AI for cows that ovulated after G1 compared to those not ovulating after G1.

The remaining presynch programs are GnRH-PGF-presynch options. Their general superiority to the standard PGF-presynch includes: (1) greater probability of ovulating a follicle in noncycling anovulatory cows—this may represent 10 to 25% of cows starting Ovsynch; (2) improved follicular synchrony for cows starting Ovsynch; (3) increased proportion of cows starting Ovsynch with a CL; and (4) facilitating more cows having moderate progesterone concentrations at G1 rather than very low or very high concentrations. Low to moderate concentrations of progesterone facilitate greater LH release and more cows ovulating in response to G1.

### Ideal Program?

If a producer solely relies on a timed AI program for first services, choose a presynchronization program that includes GnRH. It is important to note that GnRH suppresses estrus in cycling cows so “cherry picking” cows in heat in response to the GnRH-PGF presynch may be less successful.

Including a second PGF injection in the Ovsynch scheme seems to be a good investment that improves pregnancy outcomes. The 5- or 7-day options are equally good depending on the days of the week you desire to perform inseminations and pregnancy diagnosis. It is highly recommended that producers use two PGF injections with the 5-day program or pregnancy outcomes will be severely reduced. The 5-day program offers the options for Wednesday or Thursday pregnancy diagnosis days because resynchronization programs can begin on those days, and then PGF can be administered on Monday-Tuesday or Tuesday-Wednesday, respectively, and timed AI performed on Thursdays or Friday, respectively.

**Table 1. Age and number of corpus luteum (CL) at the PGF injection and outcome of CL regression**

Age and number of CL at PGF treatment	Proportion of cows with CL regression (%)
New CL	64
Original CL	97
Original CL + new CL	92

**Table 2. One or two PGF<sub>2α</sub> (PGF) injections affects pregnancy outcomes based on corpus luteum (CL) regression and concentrations at the first GnRH treatment (G1) of a 7-day Ovsynch option<sup>1</sup>**

Item	PGF injections <sup>2</sup>	
	One	Two
	----- % (n) -----	
Cows with complete CL regression		
Low progesterone (<1.0 ng/mL) at G1	70 <sup>a</sup> (76)	96 <sup>b</sup> (74)
High progesterone (>1.0 ng/mL) at G1	89 <sup>a</sup> (236)	98 <sup>b</sup> (214)
Overall	83 <sup>a</sup> (312)	98 <sup>b</sup> (288)
Pregnancy/AI, 32 days after timed AI		
Low progesterone (<1.0 ng/mL) at G1	33 <sup>c</sup> (107)	46 <sup>d</sup> (110)
High progesterone (>1.0 ng/mL) at G1	33 (312)	37 (289)
Overall	33 <sup>c</sup> (419)	39 <sup>d</sup> (399)

<sup>ab</sup>Proportions differ (P < 0.01).

<sup>cd</sup>Proportions differ (P < 0.05).

<sup>1</sup>Adapted from Carvalho et al. (2015a).

<sup>2</sup>One injection of PGF was injected as shown in Figure 1 for the 7-day option. Two injections of PGF were injection as shown in Figure 1 for the 5-day option.

**Table 3. Effect of presence of a corpus luteum (CL) at G1 on pregnancy outcomes in Holstein dairy cows 32 days after timed AI<sup>1</sup>**

Item	Treatment (T)			P-value <sup>2</sup>		
	7D1PGF	7D2PGF	5D2PGF	T	C1	C2
	----- Pregnancy rate % (n) -----					
Overall	36 (266)	41 (268)	44 (265)	0.14	0.05	0.56
Cows with a CL at G1	38 (196)	40 (191)	43 (189)	0.51	0.35	0.49
Cows lacking a CL at G1	30 (70)	46 (77)	45 (76)	0.11	0.03	0.98

<sup>1</sup>Adapted from Santos et al. (2015).

<sup>2</sup>C1: preplanned contrast between 7D1PGF (one PGF<sub>2α</sub>) and 7D2PGF + 5D2PGF (two PGF<sub>2α</sub>) treatments. C2: preplanned contrast between 7D2PGF (7-day protocol) and 5D2PGF (5-day protocol) treatments.

**Table 4. Timing of AI and G2 injections relative to PGF<sub>2a</sub> and subsequent pregnancy outcome at d 28 to 40 after AI**

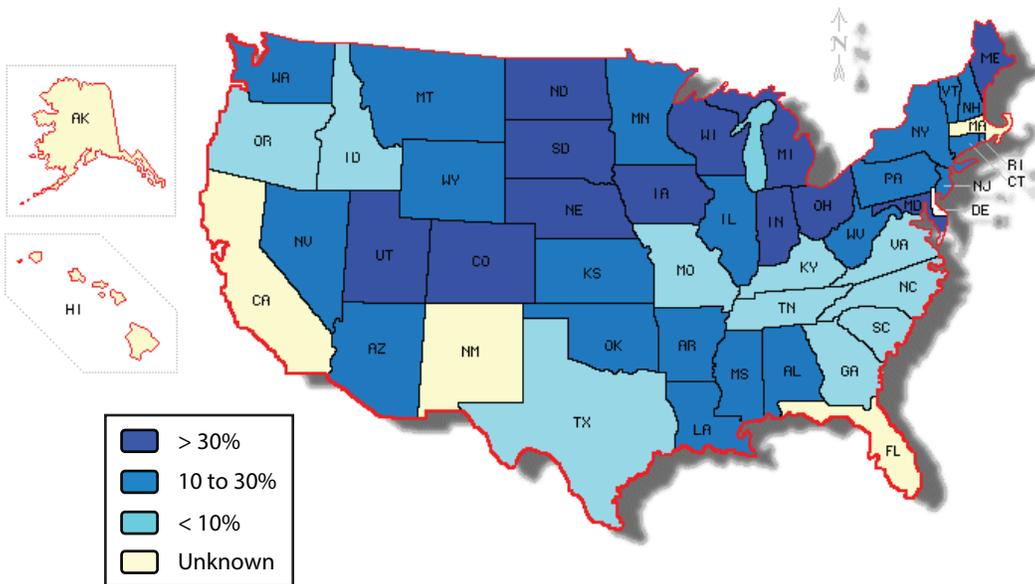
AI service	Timing of G2 and AI		
	G2 at 48 hours	G2 at 72 hours	G2 at 56 hours
	TAI at 48 hours	TAI at 72 hours	TAI at 72 hours
First services	----- % conception (n) -----		
Study average	31.3 (4) <sup>1</sup>	32.0 (4)	38.3 (3)
Weighted average	30.2 <sup>A</sup> (1,018) <sup>2</sup>	31.0 <sup>AB</sup> (870)	34.6 <sup>B</sup> (965)
Repeat services			
Study average	25.8 (2)	24.3 (3)	32.7 (2)
Weighted average	25.3 <sup>a</sup> (622)	26.5 <sup>a</sup> (712)	32.7 <sup>b</sup> (764)

<sup>A,B</sup> Percentages within row tended to differ (P = 0.08) by chi-squared test.

<sup>a,b</sup> Percentages within row with different superscript letters differ (P < 0.05) by chi-squared test.

<sup>1</sup> No. of studies.

<sup>2</sup> No. of cows.



**Figure 1. Distribution of timed AI in the United States.**

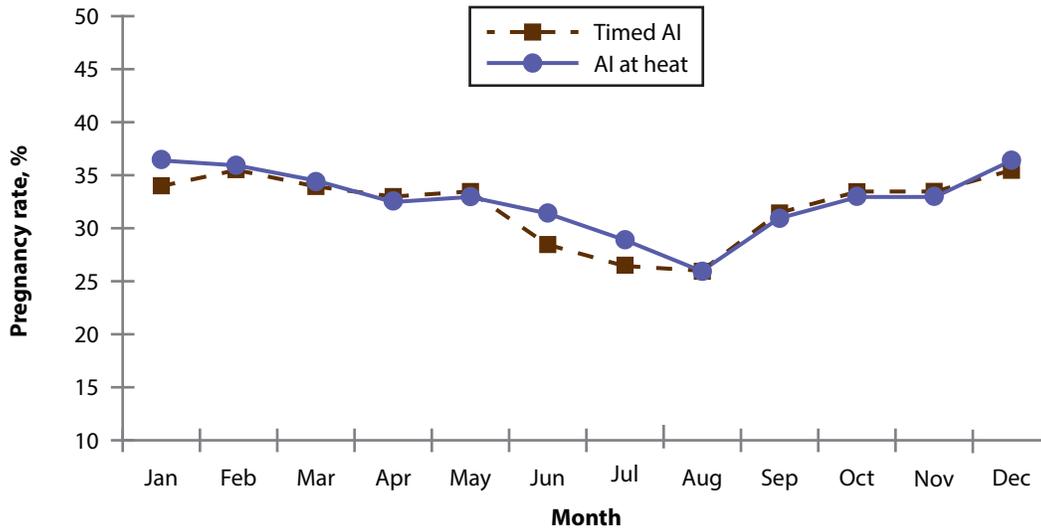


Figure 2. Pregnancy rate of lactating dairy cows during the calendar year.

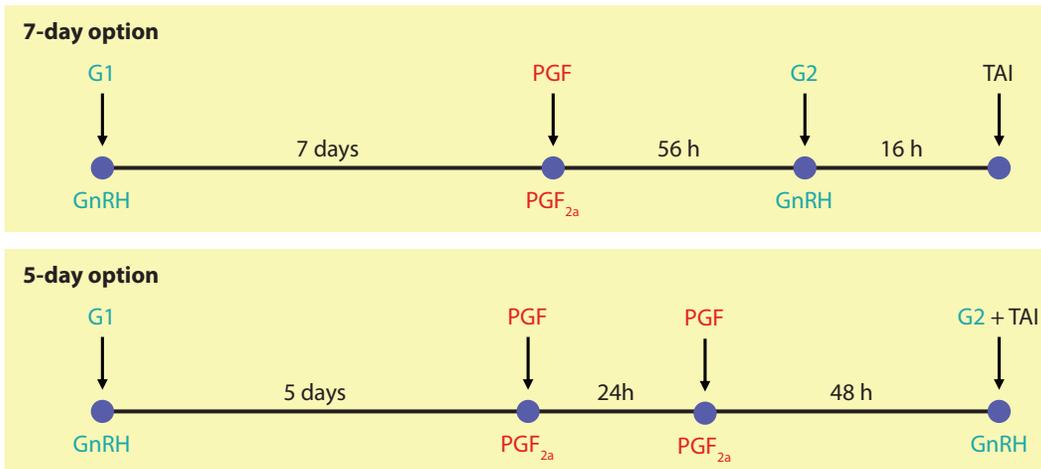


Figure 3. The standard 5- and 7-day Ovsynch timed AI programs.

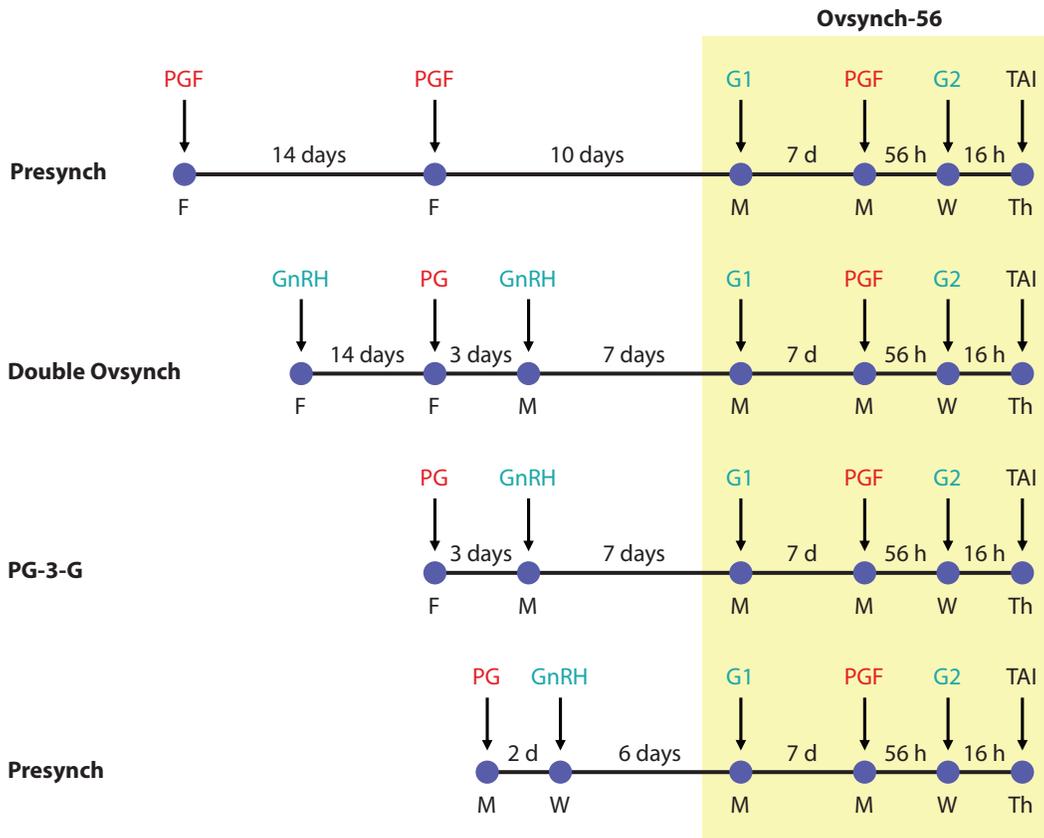


Figure 4. Examples of PGF-presynch and GnRH-PGF-presynch programs used to presynchronize estrous cycles before administering a timed AI program at first service.