Evolution of Timed AI Protocols and Overview of the 2018 DCRC Reproductive Management Strategies Protocol Sheet for Dairy Cows

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- Protocols recommended by the DCRC are periodically reviewed and updated by researchers who develop and test these protocols, and are based on the latest peer-reviewed research published in the scientific literature.
- Artificial insemination to a detected estrus plays an important role in reproductive management programs on almost all dairy farms; however, relying upon detection of estrus alone for submission of cows for first AI generally results in poor reproductive performance because of poor detection and expression of estrus.
- Presynchronization strategies that combine GnRH and $PGF_{2\alpha}$ increase P/AI by initiating ovulation in anovular cows and by increasing the proportion of cows that synchronize during an Ovsynch protocol.
- Inclusion of a second $PGF_{2\alpha}$ treatment 24 hours after the first in a 7-day Ovsynch protocol increases luteal regression thereby increasing P/AI, particularly in multiparous cows.
- Although the timing of treatments and TAI at the end of an Ovsynch protocol can ease protocol implementation and compliance, timing of AI relative to a synchronized ovulation has a profound effect on P/AI.
- Resynch protocols that include a second $PGF_{2\alpha}$ treatment 24 hours after the first can increase the risk of double-ovulation and twinning when initiated in a low-progesterone environment.
- Development and optimization of fertility programs for first and resynch TAI remains an active area of research that has advanced dramatically over the past 20 years and will most certainly change in the future.

INTRODUCTION

Synchronization protocols have been incorporated widely into reproductive management programs by most dairy farms in the U.S. (Caraviello et al., 2006; Norman et al., 2009). At first glance, it may seem that the newly released Reproductive Management Strategies for Dairy Cows protocol published by the DCRC offers many options. In reality, reproductive management strategies have generally consolidated into a few management options depending on the extent to which farms want to use artificial insemination (**AI**) to a detected estrus versus timed artificial insemination (**TAI**). It is important to clarify that there is not one "right way" to approach reproductive management on all dairy farms. Many strategies can be implemented to achieve excellent 21-day pregnancy rates by increasing the AI service rate alone (Ferguson and Skidmore, 2013). Newer fertility programs increase both service rate as well as pregnancies per artificial insemination (**P/AI**; Carvalho et al., 2018). Each individual farm must implement a plan to submit cows for first AI and to identify nonpregnant cows and return them to AI service to maximize their 21-day pregnancy rate.

Dairy farmers, dairy veterinarians, and dairy consultants are continually challenged to stay current on the latest recommendations for synchronization protocols. An excellent and up-to-date source of information on synchronization protocols can be found at the Dairy Cattle Reproduction Council (DCRC) web site:

http://www.dcrcouncil.org/

Protocols recommended by the DCRC are reviewed and updated by researchers who develop and test these protocols, and are based on the latest peer-reviewed research published in the scientific literature. The purpose of this paper is to overview the key research underlying each section of the newly updated DCRC protocol sheet for Reproductive Management Strategies for Dairy Cows released in 2018 and clarify the recommendations.

ARTIFICIAL INSEMINATION PROGRAMS

Detection of Estrus Followed by Timed AI

Artificial insemination (**AI**) to a detected estrus continues to play an important role in the overall reproductive management program on almost all dairy farms (Caraviello et al., 2006). Employing detection of estrus alone for submitting lactating dairy cows for first AI, however, generally results in poor reproductive performance because of two broad limitations associated with detection of estrus. The first limitation is the human element (i.e., visual observation and detection of estrus) in which dairy personnel must visually observe estrous behavior. Many technologies have been developed and introduced throughout the years to help overcome problems with the human element of detection of estrus. These technologies include pressure-activated heat mount devices and androgenized females (Gwazdauskas et al., 1990), tail chalking, pedometry (Peralta et al., 2005), and radiotelemetry (Walker et al., 1996; Dransfield et al., 1998). Dogs have even been trained to detect estrus-related odors in dairy cows (Kiddy et al., 1978).More recently, activity monitoring systems that use accelerometer technology to detect increased physical activity associated with behavioral estrus have been widely adopted by dairy farms.

A second limitation of detection of estrus pertains to the biology of the high-producing dairy cow. Cow-related biological factors that limit expression of estrus include the effect of high milk production on the duration of estrus (Lopez et al., 2004), ovulation failure after expression of estrus and ovulation without accompanying estrous behavior (López-Gatius et al., 2005; Valenza et al., 2012), and anovular conditions in dairy cows (Wiltbank et al., 2002). Taken together, these human-related and cow-related issues substantially limit AI service rates and 21-day pregnancy rates in dairy herds that rely on detection of estrus alone for submitting cows for AI.

A long-standing goal of reproductive biologists was to develop a hormonal synchronization protocol that would allow for TAI, thereby increasing the AI service rate. This goal was realized in 1995 with publication of the Ovsynch protocol, a synchronization protocol in which three sequential hormonal treatments are used to control ovarian function (Pursley et al., 1995). In the first field trial that evaluated the Ovsynch protocol for reproductive management (Pursley et al., 1997), lactating dairy cows managed by using only TAI 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 TAI vs. estrus, respectively) even though cows managed by using TAI were inseminated earlier postpartum. To deal with cows failing to be detected in estrus, some farms submit cows for first AI from the end of the voluntary waiting period to 80 DIM based on a detected estrus followed by submission of cows failing to be detected in estrus to an Ovsynch protocol and TAI as shown in the first section of the DCRC protocol sheet for dairy cows (Figure 1). Because of the human- and cow-related limitations to detection of estrus, all farms can increase reproductive performance by combining detection of estrus with use of Ovsynch and TAI for cows failing to be detected in estrus.

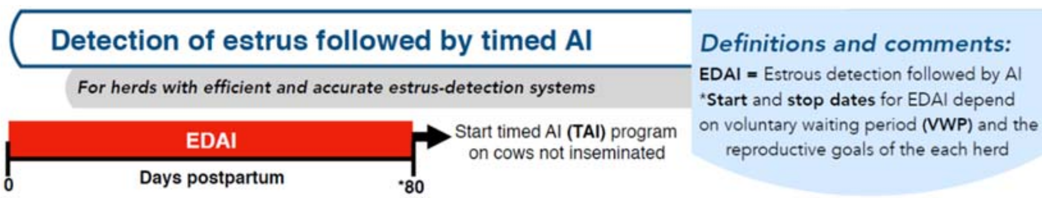

Figure 1. Applying detection of estrus to submit cows to AI before employing a TAI program to cows not detected in estrus by an elective time after calving.

Presynchronization Methods Used Before TAI

Presynchronization strategies were initially developed when it was reported that initiation of an Ovsynch protocol between days 5 to 12 of the estrous cycle resulted in more P/AI than initiation of the protocol earlier or later during the estrous cycle (Vasconcelos et al., 1999; Moreira et al., 2000; Cartmill et al., 2001). The new DCRC protocol sheet for dairy cows illustrates three options for presynchronization before initiation of an Ovsynch protocol (Figure 2).

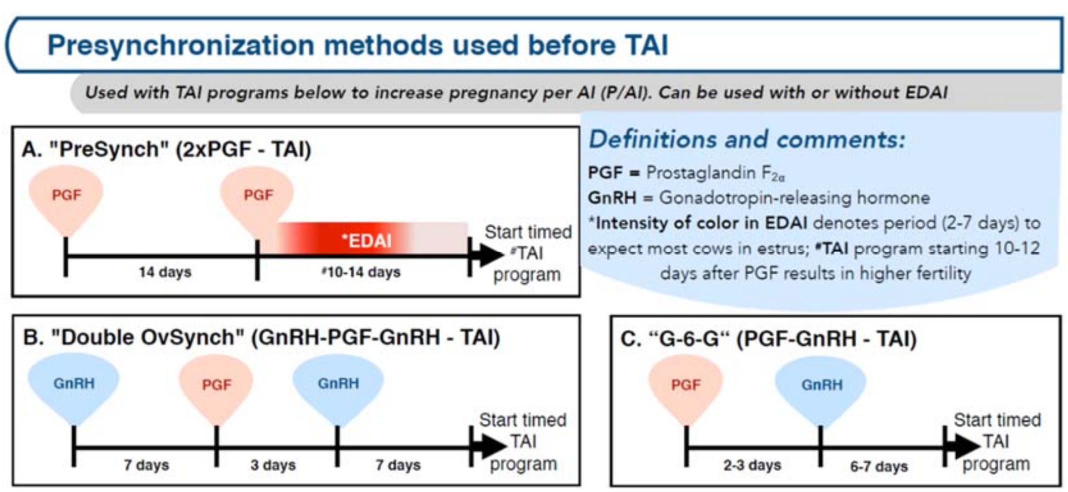

Figure 2. Presynchronization methods that can be employed before initiating a TAI program.

Presynchronization using PGF$_2$. The first presynchronization strategy tested used two $PGF_{2\alpha}$ treatments administered 14 d apart with the second $PGF_{2\alpha}$ treatment preceding the first GnRH treatment of an Ovsynch protocol by 12 days (Moreira et al., 2001; i.e., **Presynch-Ovsynch**; Figure 2A). When only cycling cows were included in the statistical analysis, P/AI to TAI increased from 29% for cows submitted to an Ovsynch protocol to 43% for cows submitted to a Presynch-Ovsynch protocol. Two things need to be clarified regarding this presynchronization strategy. First, the authors never intended that cows be inseminated to estrus during the protocol as is now commonly practiced and illustrated in Figure 2A. In fact, a recent meta-analysis of three randomized controlled studies including 1,689 cows concluded that inseminating cows that show estrus after the second $PGF_{2\alpha}$ treatment of a Presynch-Ovsynch protocol decreased P/AI compared to when all cows were allowed to complete the protocol and receive TAI (Borchardt et al., 2016). This decrease in P/AI occurs because cycling cows that are presynchronized so that the Ovsynch protocol is initiated at an optimal stage of the estrous cycle are removed from the TAI protocol thereby negating the presynchronization effect. Second, the two $PGF_{2\alpha}$ treatments preceding the Ovsynch protocol were never intended to "clean the uterus", although this effect may certainly be beneficial. An updated meta-analysis on the effect of $PGF_{2\alpha}$ therapy on bovine endometritis that included 9 experiments in 8 eligible studies and a total of 5,563 cows concluded that a positive effect on reproductive outcomes could not be verified (Haimer et al., 2018). Indeed, administration of either one or two $PGF_{2\alpha}$ treatments before initiation of a Double-Ovsynch protocol had no effect on uterine health, P/AI, or maintenance of pregnancy in lactating Holstein cows (Lima et al., 2013).

Even though the Presynch-Ovsynch protocol was originally developed to increase P/AI of cows submitted to TAI, many farms inseminate cows to a detected estrus after the second $PGF_{2\alpha}$ treatment of a Presynch-Ovsynch protocol, a practice commonly referred to as "cherry picking heats", followed by submission of cows not detected in estrus to an Ovsynch protocol (Figure 2A). Decreasing the interval between the second $PGF_{2\alpha}$ treatment of Presynch to initiation of the Ovsynch protocol from 14 to 11 d, however, increased ovulatory response to the first GnRH treatment and increased P/AI by approximately 7 percentage points when all cows were submitted to TAI (Galvão et al., 2007). Thus, if a Presynch-Ovsynch protocol is used for 100% TAI for first service, a shorter interval (i.e., 10 to 12 d) between the second $PGF_{2\alpha}$ treatment and initiation of the Ovsynch protocol is recommended. When cows were inseminated to estrus after the second $PGF_{2\alpha}$ treatment of a Presynch-Ovsynch protocol, no difference in P/AI was reported when a 12- vs. a 14-day interval was compared (Giordano et al., 2016) supporting the idea that inseminating cows to estrus during a Presynch-Ovsynch protocol negates the effect of presynchronization (Fricke et al., 2014). Furthermore, anovular cows submitted to a Presynch-Ovsynch protocol have fewer P/AI than their cycling herd mates. Because anovular cows lack a CL and therefore do not respond to the first two $PGF_{2\alpha}$ treatments of a Presynch-Ovsynch protocol, the Ovsynch protocol is initiated in a low progesterone (**P4**) environment, resulting in fewer P/AI to TAI (Carvalho et al., 2018). Because anovular cows represent 20 to 30% of cows submitted for first TAI (Bamber et al., 2009; Santos et al., 2009), presynchronization strategies using $PGF_{2\alpha}$ alone with or without inclusion of detection of estrus do not yield P/AI to TAI as those that included GnRH.

Presynchronization Strategies that Combine GnRH and $PGF_{2\alpha}$. Two limitations of a presynchronization strategy that uses $PGF_{2\alpha}$ alone are that: (1) $PGF_{2\alpha}$ does not affect anovular cows or resolve the anovular condition before initiation of the Ovsynch protocol; and (2) follicular growth is not tightly synchronized after two sequential $PGF_{2\alpha}$ treatments administered 14 d apart. Newer presynchronization strategies that combine GnRH and $PGF_{2\alpha}$ overcome both of these limitations thereby increasing P/AI to TAI. Cows that were presynchronized using an Ovsynch protocol (i.e., a **Double-Ovsynch** protocol; Figure 2B) had more P/AI than cows submitted to a Presynch-Ovsynch protocol (50% vs. 42%; Souza et al., 2008). In a subsequent study, there was a treatment by parity interaction in which the Double-Ovsynch protocol increased P/AI for primiparous, but not for multiparous cows (Herlihy et al., 2012). We now know this parity effect may occur because of incomplete luteal regression in multiparous cows (Wiltbank et al., 2015).

Presynchronization strategies have used a combination of $PGF_{2\alpha}$ (8 or 10 days before G1) and GnRH (6 or 7 days before G1) (i.e., **G6G** and **PG-3-G**), respectively (Bello et al., 2006; Stevenson and Pulley, 2012; Figure 2C). Presynchronization using a PG-3-G protocol yielded more P/AI than inseminating cows at estrus during cooler weather and was superior to a Presynch-Ovsynch 10 protocol during the summer (Stevenson and Pulley, 2012). Inclusion of GnRH into a presynchronization strategy increases P/AI to TAI by resolving the anovular condition before initiation of the Ovsynch protocol, by more tightly controlling follicular development and luteal regression, and by presynchronizing cows so that the Ovsynch protocol is initiated on either day 6 or 7 of the estrous cycle in a high proportion of cows thereby optimizing the response of cows to each sequential treatment of the Ovsynch protocol (Stevenson et al., 2012; Carvalho et al., 2018).

Synchronization Methods for TAI

This section of the DCRC Reproductive Management Strategies for Dairy Cows protocol sheet illustrates four variations of timing of treatments during an Ovsynch protocol (Figure 3). For the purposes of this discussion, the first GnRH treatment of the Ovsynch protocol will be referred to as **G1**, and the last GnRH treatment will be referred to as **G2**. A number of experiments have compared various timings of the treatments within the Ovsynch protocol as well as timing of AI relative to the last GnRH treatment of the protocol. These variations can lead to differences in P/AI, and a review of several key studies can help farms to determine which of the four variations in this section of the DCRC protocol sheet may work best for a given situation.

In the first published experiment using Ovsynch to hormonally synchronize ovulation (Pursley et al., 1995), lactating cows were submitted to TAI approximately 24 hours after the last GnRH treatment of the protocol. All cows (n = 20) ovulated to the G2 by 24 to 32 hours after G2, which is similar to the interval from the first standing event of estrus to ovulation of 27.6 hours (Walker et al., 1996). Thus, from a physiologic perspective, timing of ovulation is similar when comparing the interval from the first standing event of estrus or G2 to ovulation.

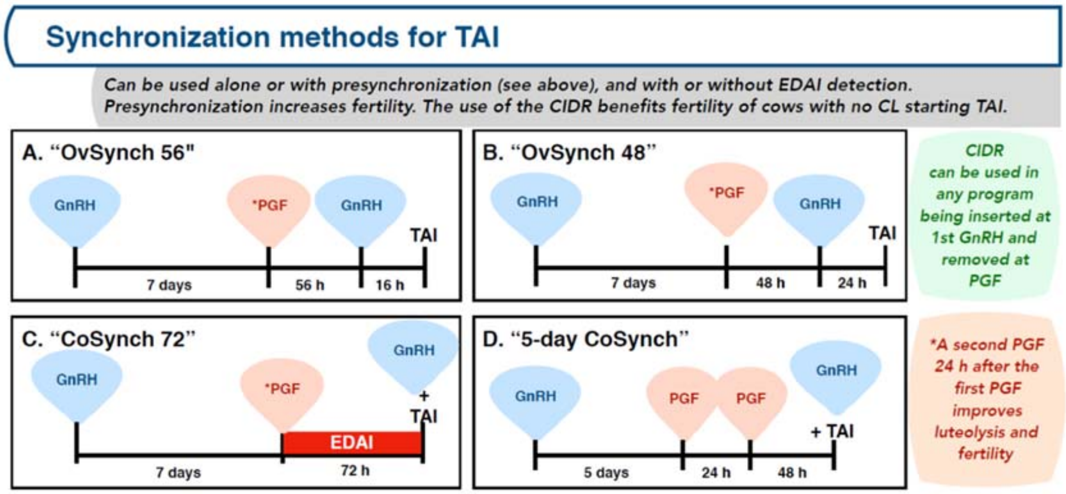

Figure 3. Timed AI options that include variations of the Ovsynch protocol,

To assess the effect of timing of AI relative to a synchronized ovulation, lactating dairy cows (n = 732) were randomly assigned to five treatments by stage of lactation and parity (Pursley et al., 1998). Ovulation was synchronized using an Ovsynch 48 protocol, and TAI was varied from 0, 8, 16, 24, or 32 hours relative to G2. In this study, the 24-hour treatment is equivalent to the **Ovsynch 48** protocol illustrated in Figure 3B. Overall, cows in the 0-, 8-, 16-, and 24-hour treatments had more P/AI than cows in the 32-hour treatment (Table 1).

Table 1. Effect of timing of AI relative to the last GnRH treatment of an Ovsynch 48 protocol on pregnancies per artificial insemination (P/AI) in lactating Holstein cows[1].

	Hours from second GnRH injection of Ovsynch (G2) to TAI					
Item	0	8	16	24	32	Total
n	149	148	149	143	143	732
P/AI (%)	37	41	45	41	32[a]	39

[1]Adapted from Pursley et al., 1998.
[a]Differs from other treatments within row ($P < 0.10$).

Thus, although no statistical difference in fertility was detected when TAI occurred from 0 to 24 hours after the last GnRH treatment of the Ovsynch protocol, inseminating too late (i.e., at 32 hours) resulted in fewer P/AI (Pursley et al., 1998). Although this study included more than 700 cows, the number of experimental units in each treatment was less than 150 cows thereby decreasing the statistical power necessary to detect differences among these treatments that may have been physiologically relevant.

To further evaluate timing of AI relative to G2, a field trial was conducted to compare two variations of a Cosynch protocol (i.e., Cosynch 48 and Cosynch 72 compared in two earlier experiments; Portaluppi and Stevenson, 2005; Sterry et al., 2007), in which TAI occurred concomitant to G2, with a variation of the Ovsynch protocol in which TAI occurred 16 hours after G2 (Brusveen et al., 2008). This third treatment is now referred to as the **Ovsynch 56** protocol (Figure 3A). Timing of AI in the Ovsynch 56 protocol is supported by the data in Table 1 in which the 16-hour interval from the last GnRH treatment to TAI resulted in numerically (but not statistically) greater fertility than the other treatments as well as data reporting that optimal fertility should occur when cows are inseminated around 15 to 24 hours before ovulation (Walker et al., 1996; Dransfield et al., 1998). Because timing of ovulation is similar when comparing the interval to ovulation from the first standing event of estrus or G2, timing of AI based on a Cosynch protocol will not optimize timing of AI relative to an induced ovulation.

Most farms using the Ovsynch 56 protocol administer G1, the $PGF_{2\alpha}$ treatment, and TAI in the morning, whereas G2 is administered in the afternoon to achieve a 56-hour interval from the $PGF_{2\alpha}$ treatment to G2 and a 16-hour interval from G2 to TAI

Despite the data in Table 2 supporting that an Ovsynch 56 protocol yielding more P/AI, it is difficult for some farms to implement this timing of treatments because of the inconvenience or inability to handle cows in the afternoon. Most of these farms prefer the timing of the **Ovsynch 48** protocol (Figure 3B) or a **Cosynch 72** protocol (Figure 3C). Thus, the Ovsynch variations illustrated in Figure 3B and 3C are based on ease of implementation on farms rather than biology. Because of the extended interval between G2 and TAI in the Cosynch 72 protocol, many cows will display estrus more than 12 hours before scheduled TAI, thereby decreasing fertility to TAI (Brusveen et al., 2008). As illustrated in Figure 3C, detection of estrus and AI between the $PGF_{2\alpha}$ treatment and G2 of the Cosynch 72 protocol may help mitigate the decreased fertility to TAI when using this protocol variation.

Table 2. Effect of treatment on pregnancies per artificial insemination (P/AI) and pregnancy loss in lactating Holstein cows[1].

Item	Cosynch 48	Ovsynch 56	Cosynch 72
P/AI 31-33 d, % (n)	27 (494)	36 (457)	27 (517)
Least squares estimate	29^{a}	39^{b}	25^{a}
P/AI 52-54 d, % (n)	25 (493)	33 (450)	25 (513)
Least squares estimate	27^{a}	36^{b}	23^{a}
Pregnancy loss, % (n)	5 (131)	5 (158)	7 (137)

[1]Adapted from Brusveen et al. (2008).

[a,b]Proportions with different superscripts differ ($P < 0.05$).

The last option in this section illustrates a **5-day Cosynch** protocol (Figure 3D) in which the interval between G2 and the $PGF_{2\alpha}$ treatment is decreased from 7 (7-day protocol) to 5 (5-day protocol) days. The 5-day Cosynch protocol was first reported in a series of experiments in beef cows (Bridges et al., 2008). Although timing of AI after the $PGF_{2\alpha}$ treatment differed between cows in the 7-day protocol than in the 5-day protocol, cows submitted to the 5-day protocol had more P/AI than cows submitted to the 7-day protocol in two experiments (80% vs. 67%, respectively and 65% vs. 56%, respectively). In 2010, the 5-day Ovsynch protocol was compared to a 7-day Cosynch 72 protocol in lactating Holstein cows (Santos et al., 2010). In that study, cows submitted to the 5-day protocol received two $PGF_{2\alpha}$ treatments, whereas cows submitted to the 7-day protocol received only a single $PGF_{2\alpha}$ treatment. Overall, cows in the 5-day protocol had more P/AI than cows in the 7-

day protocol (38% vs. 31%). The authors conducted an analysis to control for a difference in luteal regression rates between cows receiving one vs. two $PGF_{2\alpha}$ treatments by analyzing only cows with P4 < 1 ng/mL on the day of TAI, and cows submitted to the 5-day protocol had more P/AI than cows submitted to the 7-day protocol (39% vs. 34%). The authors attributed this treatment effect to the decreased period of follicle dominance for cows in the 5-day Cosynch protocol. Colazo and Ambrose (2015) also compared a 5-day Cosynch protocol with two $PGF_{2\alpha}$ treatments to a 7-day Ovsynch protocol with one $PGF_{2\alpha}$ treatment; however, P/AI did not differ between treatments in that study (39% vs. 34%).

A recent experiment directly tested the effect of addition of a second $PGF_{2\alpha}$ treatment and the effect of decreasing the duration of the Ovsynch protocol from 7 to 5 days in a Resynch protocol (Santos et al., 2016). Lactating Holstein cows (n = 821) were assigned randomly at a nonpregnancy diagnosis (d 0 = 32 d after AI) to one of three Resynch protocols: (1) 7D1PGF (GnRH, d 0; $PGF_{2\alpha}$, d 7; GnRH, d 9.5); (2) 7D2PGF (GnRH, d 0; $PGF_{2\alpha}$, d 7; $PGF_{2\alpha}$, d 8; GnRH, d 9.5); and (3) 5D2PGF (GnRH, d 2; $PGF_{2\alpha}$, d 7; $PGF_{2\alpha}$, d 8; GnRH, d 9.5). All cows received an intravaginal P4 insert (PRID Delta; Ceva Santé Animale, Libourne, France) at G1, which was removed at the first $PGF_{2\alpha}$ treatment, and all cows received a TAI approximately 16 hours after G2. Overall, no effect of treatment on P/AI was detected (Table 3). When these data were analyzed based on the presence or absence of a CL at G1, cows lacking a CL and receiving two $PGF_{2\alpha}$ treatments had more ($P = 0.03$) P/AI than cows receiving one $PGF_{2\alpha}$ treatment regardless of protocol duration (i.e., 5 vs. 7 d), whereas there was no effect of treatment for cows that had a CL at G1 (Table 3). We concluded that addition of a second $PGF_{2\alpha}$ treatment to a Resynch protocol increased the proportion of cows with CL undergoing complete luteal regression, thereby increasing P/AI, particularly for cows that have low P4 at G1, whereas decreasing the duration of the Ovsynch protocol did not affect P/AI. Nonetheless, the 5-day Cosynch protocol is a good option for dairy farms that want to administer all protocol treatments and TAI in the morning, thereby simplifying implementation of this protocol. In addition, the 5-day protocol allows for nonpregnancy diagnoses on Wednesday and Thursday so that cows diagnosed not pregnant can be reinseminated on the following Thursday or Friday.

Table 3. Effect of presence of a corpus luteum (CL) at Day 0 on pregnancies per AI (P/AI) in Holstein dairy cows 32 days after TAI[1].

	Treatment			*P*-value[2]		
Item	7D1PGF	7D2PGF	5D2PGF	T	C1	C2
	--------------------P/AI, % (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

[1]Adapted from Santos et al. (2016).

[2]C1: preplanned contrast between 7D1PGF (one $PGF_{2\alpha}$) and 7D2PGF + 5D2PGF (two $PGF_{2\alpha}$) treatments; C2: preplanned contrast between 7D2PGF (7-day protocol) and 5D2PGF (5-day protocol) treatments.

Inclusion of a Second $PGF_{2\alpha}$ Treatment 24 hours after the First in Ovsynch Protocols

A major change in the new DCRC Reproductive Management Strategies for Dairy Cows protocol sheet is the recommendation to include a second $PGF_{2\alpha}$ treatment 24 hours after the first in the 7-day Ovsynch protocol variations illustrated in Figure 3. Inclusion of a second $PGF_{2\alpha}$ treatment is absolutely necessary for the 5-day Cosynch protocol (Figure 3D) because when a new younger CL forms after G1 and is present at the $PGF_{2\alpha}$ treatment, it will fail to regress after a single $PGF_{2\alpha}$ treatment (Nascimento et al., 2014; Stevenson et al., 2014). Addition of a second $PGF_{2\alpha}$ treatment is highly recommended for all of the 7-day protocols illustrated in Figure 3A, 3B, and 3C, particularly when used for first TAI after a presynchronization strategy that incorporates both GnRH and $PGF_{2\alpha}$ (i.e., Figure 2B and 2C). Lack of complete luteal regression particularly for multiparous cows, which is addressed by the addition of the second $PGF_{2\alpha}$ treatment, was in fact, the rate limiting factor for fertility to TAI (Wiltbank et al., 2015). Indeed, submission of lactating Holstein cows to a Double-Ovsynch protocol and TAI for first insemination increased the percentage of cows inseminated by 7 days after the end of the voluntary waiting period 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 range of days in milk (Santos et al., 2017).

Several experiments have been conducted to assess the addition of a second $PGF_{2\alpha}$ treatment on luteal regression and P/AI (Brusveen et al., 2009; Wiltbank et al., 2015; Barletta et al., 2018; Stevenson et al., 2018). A recent meta-analysis of data from these experiments was conducted with the primary objective to evaluate the effect of an additional $PGF_{2\alpha}$ treatment during the Ovsynch protocol on luteal regression and P/AI (Borchardt et al., 2018). The meta-analysis included seven randomized controlled experiments from 6 published manuscripts including 5,356 cows, and information regarding luteal regression at the end of the Ovsynch protocol was available for 1,856 cows. Including a second $PGF_{2\alpha}$ treatment 24 hours after the first during the Ovsynch protocol increased the relative risk (**RR**) of complete luteal regression at the end of the Ovsynch protocol (RR = 1.14; 95% confidence interval = 1.10 to 1.17) using a fixed-effects model and the RR for pregnancy (RR = 1.14; 95% confidence interval = 1.06 to 1.22) at 32 days after TAI. No heterogeneity was observed among the 6 manuscripts regarding complete luteal regression and P/AI. The authors concluded that there was a clear benefit of including an additional $PGF_{2\alpha}$ treatment during the Ovsynch protocol on luteal regression (+11.6 percentage units) and on P/AI (+4.6 percentage units). Inclusion of a second $PGF_{2\alpha}$ treatment in 7-day Ovsynch protocols is now recommended by the DCRC. Finally, in at least one experiment, the increase in P/AI from inclusion of a second $PGF_{2\alpha}$ treatment was primarily manifest in multiparous cows (Wiltbank et al., 2015).

Although addition of a second $PGF_{2\alpha}$ treatment to Ovsynch protocols increases luteolysis and P/AI, it also increases the number of times cows have to be handled. A common question is whether increasing the dose of $PGF_{2\alpha}$ at a single time can achieve a similar rate of luteolysis and/or P/AI as including a second $PGF_{2\alpha}$ treatment. Two prostaglandin products are available and approved for use in dairy cows in the U.S.: dinoprost (i.e., native $PGF_{2\alpha}$) and cloprostenol (a $PGF_{2\alpha}$ analog). Doubling the dose of dinoprost from 25 to 50 mg does not seem to perform as well as two 25-mg dinoprost treatments administered 24 hours apart for first TAI in a 5-day protocol (Stevenson et al., 2018) or Resynch TAI in a 7-day protocol (Barletta et al., 2018) TAI. Increasing the dose of cloprostenol from 500 to 750 µg increased the incidence of luteal regression primarily in multiparous cows, but tended to increase fertility ($P = 0.05$) only at the pregnancy diagnosis 39 days after TAI (Giordano et al., 2013). Finally, delaying a single dinoprost treatment by 24 hours (i.e., from day 7 to day 8 of the protocol) without adjusting G2 and TAI decreased luteal regression and P/AI (Niles et al., 2017). Because of the complexity of much of the data generated thus far, more studies are needed to definitively answer this question using both prostaglandin products. At the present time, the new DCRC recommendation of adding a second $PGF_{2\alpha}$ treatment 24 hours after the first to both 7 d and 5 d Ovsynch protocols should be followed.

Resynchronization Programs

The new DCRC Reproductive Management Strategies for Dairy Cows protocol sheet illustrates three options for Resynchronization programs (Figure 4).

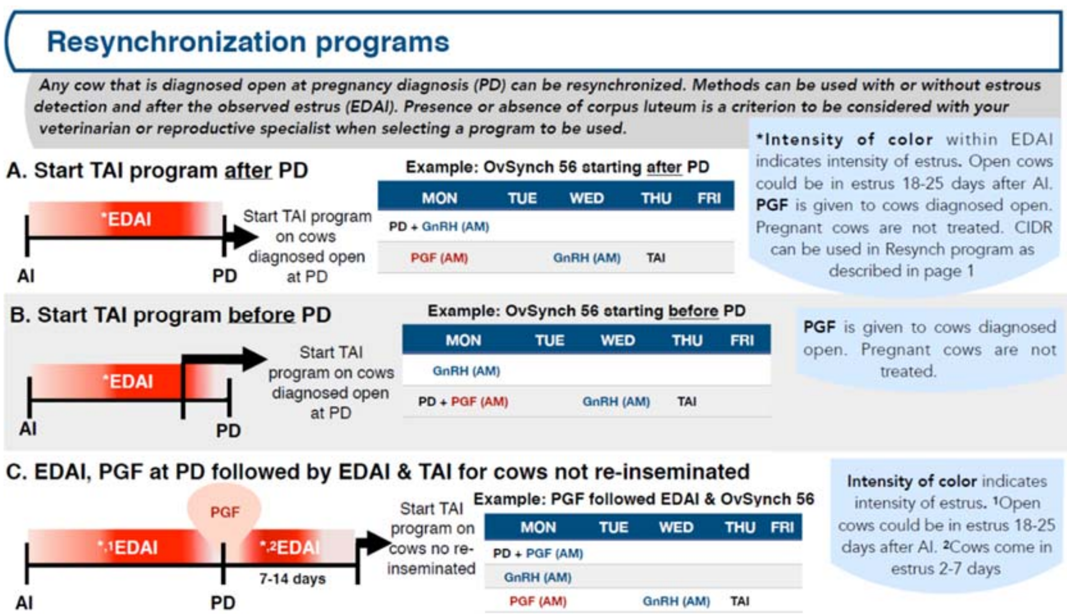

Figure 4. Recommended options for resynchronization programs associated with a nonpregnant diagnosis.

Although all three options include detection of estrus and AI after an initial AI, some farms choose to minimize use of AI to estrus and submit nearly all cows to TAI. In this management scenario, the AI service rate is fixed based on the interval between inseminations which is set by the timing of pregnancy diagnosis, and the primary emphasis is focused on compliance to the protocols, a key element to their success (refer to compliance table on the DCRC protocol sheet). Nonetheless, including detection of estrus after an initial AI can increase 21-day pregnancy rates by increasing the AI service rate. Farm managers should keep in mind that they must manage two reproductive management systems in this scenario; one for the TAI protocol, and the other for the daily chore of detection of estrus and AI. Nonetheless, most of the DCRC award-winning dairy herds in 2017, which all had annualized 21-day pregnancy rates between 30% and 40%, submitted all cows to TAI after a fertility program, inseminated any cows detected in estrus after first TAI, and then submitted cows not detected in estrus and diagnosed not pregnant to a Resynch protocol.

Return to Estrus after AI

Accurate detection of cows failing to conceive to AI and returning to estrus from 18 to 32 d after AI is the earliest method for identifying and reinseminating cows failing to conceive after AI. There are, however, several challenges for detection of estrus after AI. First, only, 52% of the eligible cows were detected in estrus and re-inseminated between AI and pregnancy diagnosis when detection of estrus was performed through continuous monitoring of activity after a previous AI until pregnancy diagnosis 32 d after AI (Giordano et al., 2015). Second, estrus-cycle duration varies widely with a high degree of variability among individual cows (Remnant et al., 2015). Finally, the high rate of early pregnancy losses in dairy cows increases the interval from insemination to return to estrus for cows that establish pregnancy early then undergo pregnancy loss (Ricci et al., 2017). Because of these issues with nonpregnant cows returning to estrus, implementation of a Resynch strategy is critical for achieving high 21-day pregnancy rates.

Timing of Pregnancy Diagnosis and Initiation of Resynch

Figure 4 illustrates two options for timing of pregnancy diagnosis and initiation of a Resynch protocol. Figure 4A illustrates pregnancy diagnosis before initiation of the Resynch protocol, whereas Figure 4B illustrates administering the first GnRH treatment of a Resynch protocol 7 d before pregnancy diagnosis. Choosing between these two Resynch variations depends on the reproductive management goals of the dairy farm. The advantage of delaying G1 until the pregnancy diagnosis is that more time is allowed for cows to show estrus for submission to AI thereby decreasing the total number of cows submitted to a Resynch protocol (Bruno et al., 2014). For herds focused on detecting cows in estrus and minimizing cows submitted to TAI, this is a good option. The disadvantage of this approach is that the Resynch protocol is delayed by one week because of the need to identify nonpregnant cows before G1. The obvious disadvantage of administering G1 before pregnancy diagnosis is that all cows are treated with GnRH regardless of their pregnancy status, which is unknown at the time of treatment. Herds that have excellent detection of estrus after an AI have a large proportion of cows diagnosed pregnant at the herd check, and these cows are unnecessarily treated with GnRH. By contrast, one advantage of administering G1 before pregnancy diagnosis is that TAI occurs one week earlier. Overall, P/AI did not differ between cows submitted to a Resynch protocol 32 or 39 d after AI (Lopes et al., 2013), so the earlier Resynch protocol decreases the interval between TAI services thereby increasing the AI service rate. A second advantage of administering G1 before pregnancy diagnosis is that management decisions can be made based on the presence or absence of a CL at the $PGF_{2\alpha}$ treatment of the Ovsynch protocol (see the next section).

The third Resynch strategy in this section of the DCRC protocol sheet is depicted in Figure 4C. This strategy is based on decreasing the interval between AI services by increasing the AI service rate. Cows presynchronized with a single $PGF_{2\alpha}$ treatment administered 12 days before G1 of a Resynch protocol had more P/AI for the cows with high P4 (>1.0 ng/mL) at G1 (Silva et al., 2007). When cows were presynchronized with either GnRH or $PGF_{2\alpha}$ 7 d before G1of a Resynch protocol, more cows were inseminated to estrus when a $PGF_{2\alpha}$ treatment was added, whereas more cows completed the Resynch protocol when a GnRH treatment was added (Bruno et al., 2013). Thus, the Resynch strategy illustrated in Figure 4C is a good option for herds that wish to maximize AI to estrus and minimize TAI.

Presence or Absence of a CL at Initiation of the Ovsynch Protocol and Fertility to Resynch

Based on P4 profiles at each treatment during the Ovsynch protocol, the best indicator of poor fertility to TAI is low P4 (i.e., cows lacking a functional CL) at the $PGF_{2\alpha}$ treatment of the Ovsynch protocol (Carvalho et al., 2018). One of the first strategies to increase P/AI to a Resynch protocol attempted to determine the optimal interval after an initial TAI to initiate G1 based on the physiology of the estrous cycle (Fricke et al., 2003). Assuming an estrous cycle duration of 21 to 23 days, administering G1 32 days after AI should correspond to initiating the Resynch protocol approximately day 6 to 14 of the estrous cycle, a stage of the estrous cycle when a dominant follicle may have turned over before G1, thus resulting in failure of ovulation to G1. Cows identified not pregnant 32 d after AI with a CL at G1 have more P/AI than cows without a CL (Giordano et al., 2012; Lopes et al., 2013). In several studies however, 16%, 22%, and 35% of cows diagnosed not pregnant 32 d after TAI and that were not presynchronized with GnRH 7 d before pregnancy diagnosis lacked a CL at G1 (Fricke et al., 2003; Giordano et al., 2015). When cows were synchronized for first TAI and P4 profiles and CL diameter was measured until a pregnancy diagnosis 32 days later, 19% of cows diagnosed not pregnant lacked a CL > 10 mm in diameter (Ricci et al., 2017). Thus, Resynch protocols are initiated in a low-P4 environment in up to one-third of nonpregnant cows which leads to a lack of complete luteal regression after treatment with $PGF_{2\alpha}$ 7 d later resulting in fewer P/AI. Inclusion of a second $PGF_{2\alpha}$ treatment 24 hours after the first into a Resynch protocol increases P/AI for cows initiating Resynch in a low-P4 environment (Carvalho et al., 2015).

One strategy to treat nonpregnant cows without a CL at G1 is to supplement with exogenous P4 during the Resynch protocol. Cows without a CL at G1 and treated with a CIDR insert for 7 days had more P/AI at first as well as Resynch TAI (Stevenson et al., 2008; Chebel et al., 2010; Bilby et al., 2013; Bisinotto et al., 2015). Many veterinarians now use the presence or absence of a CL at a nonpregnancy diagnosis to implement a strategy to increase fertility to Resynch protocols or to increase the proportion of cows inseminated to a detected estrus after AI. Based on this idea, a recent study assigned cows diagnosed not pregnant to various Resynch strategies based on ovarian structures (Wijma et al., 2018). The control treatment was a standard Resynch protocol in which G1 was administered 32 d after AI and including a single $PGF_{2\alpha}$ treatment. Alternatively, cows diagnosed not pregnant 32 d after AI were assigned to a Resynch strategy based on the presence or absence of a CL >15 mm in diameter. Nonpregnant cows with a CL received two $PGF_{2\alpha}$ treatments 24 hours apart followed by GnRH and TAI (i.e., a Resynch protocol without G1), whereas nonpregnant cows without a CL were submitted to a Resynch protocol that included a second $PGF_{2\alpha}$ treatment and a CIDR insert. It is important to note that cows were detected in estrus and inseminated from the initial AI to initiation of each of the three

Resynch treatments. The authors concluded that the shorter Resynch program decreased time to pregnancy because of a decrease in the interval between AI services for nonpregnant cows with a CL and more P/AI in nonpregnant cows lacking a CL (Wijma et al., 2018). This Resynch strategy is a good option for herds that combine detection of estrus after first TAI with a Resynch strategy.

Herds that do not incorporate detection of estrus after an initial TAI can implement a Resynch strategy based on ovarian structures as described by Carvalho et al. (2018). In this strategy, all cows are treated with GnRH 25 d after TAI. Pregnancy diagnosis is conducted using transrectal ultrasonography 32 d after TAI, and cows diagnosed not pregnant are classified as having or lacking a CL. Nonpregnant cows with a CL continue an Ovsynch 56 protocol by receiving a $PGF_{2\alpha}$ treatment 32 d after TAI with the addition of a second $PGF_{2\alpha}$ treatment 24 hours after the first. Nonpregnant cows lacking a CL restart an Ovsynch 56 protocol that includes a second $PGF_{2\alpha}$ treatment 24 hours after the first (i.e., GGPPG) as described by Carvalho et al. (2015). Intravaginal P4 inserts (one per cow) are included as part of the Ovsynch protocol for cows without a CL based on studies in which treatment with exogenous P4 increased P/AI for cows lacking a CL at initiation of an Ovsynch protocol to that of cows with a CL at initiation of an Ovsynch protocol (Bilby et al., 2013; Bisinotto et al., 2015).

Low Progesterone, Double-Ovulations, and Twinning: A New Problem with Protocols

Low P4 during growth of an ovulatory follicle is associated an increased incidence of double ovulation (Wiltbank et al., 2000). Cows in which the preovulatory follicle develops in the absence of P4 from a CL have a greater incidence of co-dominant follicles resulting in double ovulations (Stevenson et al., 2007; Hayashi et al., 2008). All dairy cows experience a low P4 environment during the postpartum anovular period from calving to first ovulation. Double ovulation incidence after a spontaneous estrus was greater for anovular cows (i.e., low P4) than for cycling cows (Lopez et al., 2005). Incidence of double ovulation to G1 was greater for anovular than for ovular cows; however, incidence of double ovulation to G2 was similar between ovular and anovular cows (Gumen et al., 2003). Thus, the first postpartum ovulation results in a high incidence of double ovulation because of the lack of P4 during growth of the preovulatory follicle, and the first exposure to P4 during the postpartum anovular period decreases the incidence of double ovulation.

To test the effect of P4 during growth of the ovulatory follicle on the incidence of double ovulation, Holstein cows were assigned randomly to two presynchronization protocols that manipulated cows into either a high or a low P4 environment during an Ovsynch protocol (Cunha et al., 2008; Table 4). Cows in the high P4 treatment were submitted to a Double-Ovsynch protocol (Souza et al., 2008) and had more P4 at the first GnRH treatment of the Ovsynch protocol and at the $PGF_{2\alpha}$ treatment of the Ovsynch protocol than cows in the low P4 treatment. Ovulatory response to G2 was similar between treatments; however, cows in the low P4 treatment, had more double ovulations than cows in the high P4 treatment. Furthermore, fertility was greater and pregnancy loss was less for cows in the high vs. the low P4 treatment. Thus, cows with high P4 during growth of the ovulatory follicle had fewer double ovulations, more P/AI, and fewer pregnancy losses than cows with low P4.

Table 4. Effect of progesterone during growth of the preovulatory follicle on incidence of double ovulation in Holstein dairy cows[1]

Item	Low progesterone (n = 259)	High progesterone (n = 255)	*P*-value
P4 at 1st GnRH (ng/mL)	0.28	1.84	…
P4 at $PGF_{2\alpha}$ (ng/mL)	2.23	4.40	…
Ovulation to G2 (%)	95	95	NS
Double Ovulation (%)	21	7	<0.05
P/AI at 29 d (%)	33	48	<0.01
Pregnancy loss 29 to 57 d (%)	16	4	<0.05

[1]Adapted from Cunha et al. (2008).

It is important to note that the study by Cunha et al. (2008) was conducted before the second $PGF_{2\alpha}$ treatment was included in the Ovsynch protocol. Therefore, we must now interpret these data based on a current understating of the physiology associated with these protocols in which a lack of complete luteal regression decreases P/AI. Thus, in the study by Cunha et al. (2008), cows in the low P4 treatment had a high incidences of double ovulation, but low conception rates resulting from incomplete luteal regression. For cows that initiate an Ovsynch protocol in a low-P4 environment, if the luteal regression problem is partly overcome by adding a second $PGF_{2\alpha}$ treatment, P/AI may increase because of increased double ovulations (Fricke and Wiltbank, 1999) followed by increased pregnancy losses for cows that conceive unilateral twins (López-Gatius and Hunter, 2004), followed by an increase in twins for cows that maintain the twin pregnancy. Thus, a new problem has arisen concurrent with the recommendation to add the second $PGF_{2\alpha}$ treatment to Ovsynch protocols particularly when cows initiate the protocol in a low P4 environment.

To further evaluate the effect of manipulating P4 before TAI, lactating Holstein cows (n = 80) were synchronized for first TAI using a Double-Ovsynch protocol that included a second $PGF_{2\alpha}$ treatment 24 hours after the first and were assigned randomly to receive 25 mg $PGF_{2\alpha}$ 1 day after G1 that included a used CIDR insert (Low P4) or to receive 2 new CIDR inserts during the breeding Ovsynch protocol (High P4). Results of this experiment are shown in Table 5.

Table 5. Effect of progesterone during growth of the preovulatory follicle on follicle size, incidence of double ovulation, pregnancies per artificial insemination (P/AI), and twin pregnancies in Holstein dairy cows[1]

Item	Low progesterone (n = 40)	High progesterone (n = 40)	*P*-value
Follicle size at G2 (mm)	16.4 ± 5	14.8 ± 0.3	< 0.01
Double ovulation (%)	33	10	< 0.01
P/AI at 32 d (%)	45	53	0.97
Twins at 32 d (%)	0	29	< 0.01

[1]Carvalho al., unpublished.

Incidence of double ovulation was three-fold greater for Low P4 than for High P4 cows. Overall, P/AI at 32 d did not differ between treatments; however, Low P4 cows had more twin pregnancies than High P4 cows. We concluded that low P4 concentrations before TAI increased the incidence of double ovulations and twin pregnancies. The data in Table 5 agree with a larger study in which cows were manipulated into high vs. low P4 environments during growth of the ovulatory follicle (Martins et al., 2018). In that study, cows that were maintained in a low P4 environment during growth of the ovulatory follicle had a double ovulation rate of 49%, P/AI of 66.4%, and pregnancy loss from 23 to calving of 33% (Martins et al., 2018).

To summarize, the problem with the increased risk of double ovulation and twinning occurs when cows are submitted to an Ovsynch protocol that includes a second $PGF_{2\alpha}$ treatment and initiate the protocol a low-P4 environment. This scenario also leads to increased pregnancy losses resulting from bilateral twins (Martins et al., 2018), and may explain a significant proportion of pregnancy losses that occur in dairy herds. There are two primary management scenarios under which this scenario arises. The first scenario is when herds that use a Presynch-Ovsynch protocol for first AI include detection of estrus after the second $PGF_{2\alpha}$ treatment of the protocol. When an activity-monitoring system was used, approximately 70% of cows were inseminated to increased activity after the second $PGF_{2\alpha}$ treatment of a Presynch-Ovsynch protocol, and about half of the cows not detected with increased activity had low-P4 at the first GnRH treatment of the Ovsynch protocol (Fricke et al., 2014). This scenario can be avoided by using a presynchronization strategy that combines both GnRH and $PGF_{2\alpha}$ (Figure 2B and 2C) because these presynchronization strategies set up a larger proportion of cows to have a CL at G1. A second scenario arises when herds submit cows without a CL either knowingly or unknowingly to a Resynch protocol that includes a second $PGF_{2\alpha}$ treatment. This scenario can be avoided by submitting cows to the Resynch protocol based on ovarian structures with the nonpregnant cows lacking a CL treated with a CIDR insert which should increase P4 during the protocol and decrease the incidence of double ovulation.

CONCLUSIONS

Development and optimization of fertility programs for first and resynch TAI remains an active area of research that has advanced dramatically over the past 20 years and will most certainly change in the future. It takes time for researchers to sift and winnow ideas and data to reach a consensus on protocols to recommend for use on commercial dairy farms. Scientific progress holds the potential to change longstanding recommendations. An excellent and up-to-date source of information on synchronization protocols can be found at the Dairy Cattle Reproduction Council (DCRC) web site:

http://www.dcrcouncil.org/

REFERENCES

Bamber R. L., G. E. Shook, M. C. Wiltbank, J. E. P. Santos, and P. M. Fricke. 2009. Genetic parameters for anovulation and pregnancy loss in dairy cattle. J. Dairy Sci. 92:5739-5753.

Barletta, R. V., P. D. Carvalho, V. G. Santos, L. F. Mello, C. E. Consentini, A. S. Netto, and P. M. Fricke. 2018. Effect of dose and timing of prostaglandin $F_{2\alpha}$ treatments during a Resynch protocol on luteal regression and fertility in lactating Holstein cows. J. Dairy Sci. 101:1730–1736.

Bello, N. M., J. P. Steibel, and J. R. Pursley. 2006. Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. J. Dairy Sci. 89:3413-3424.

Bilby, T. R., R. G. S. Bruno, K. J. Lager, R. C. Chebel, J. G. N. Moraes, P. M. Fricke, G. Lopes, Jr., J. O. Giordano, J. E. P. Santos, F. S. Lima, S. L. Pulley, and J. S. Stevenson. 2013. Supplemental progesterone and timing of resynchronization on pregnancy outcomes in lactating dairy cows. J. Dairy Sci. 96:7032-7042.

Bisinotto, R. S., L. O. Castro, M. B. Pansani, C. D. Narciso, N. Martinez, L. D. P. Sinedino, T. L. C. Pinto, N. S. Van de Burgwal , H. M. Bosman, R. S. Surjus, W. W. Thatcher, and J. E. P. Santos. 2015. Progesterone supplementation to lactating dairy cows without a corpus luteum at initiation of the Ovsynch protocol J. Dairy Sci. 98:2515-2528.

Borchardt, S., P. Haimerl, and W. Heuwieser. 2016. Effect of insemination after estrous detection on pregnancy per artificial insemination and pregnancy loss in a Presynch-Ovsynch protocol: A meta-analysis. J. Dairy Sci. 99:2248-2256.

Borchardt, S., A. Pohl, P. D. Carvalho, P. M. Fricke, and W. Heuwieser. 2018. Short communication: Effect of adding a second prostaglandin $F_{2\alpha}$ injection during the Ovsynch protocol on luteal regression and fertility in lactating dairy cows: A meta-analysis. J. Dairy Sci. 101:8566–8571.

Bridges, G. A., L. A. Helser, D. E. Grum, M. L. Mussard, C. L. Gasser, and M. L. Day. 2008. Decreasing the interval between GnRH and $PGF_{2\alpha}$ from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. Theriogenology 69:843-851.

Bruno, R. G. S., A. M. Garias, J. A. Hernandez-Rivera, A. E. Navarrette, D. E. Hawkings, and T. R. Bilby. 2013. Effect of gonadotropin-releasing hormone or prostaglandin $F_{2\alpha}$-based estrus synchronization programs for first or subsequent artificial insemination in lactating dairy cows. J. Dairy Sci. 96:1556-1567.

Bruno, R. G. S., J. G. N. Moraes, J. A. H. Hernández-Rivera, K. J. Lager, P. R. B. Silva, A. L. A. Scanavez, L. G. D. Mendonça, R. C. Chebel, and T. R. Bilby. 2014. Effect of an Ovsynch56 protocol initiated at different intervals after insemination with or without a presynchronizing injection of gonadotropin-releasing hormone on fertility in lactating dairy cows. J. Dairy Sci. 97:185-194.

Brusveen, D. J., A. P. Cunha, C. D. Silva, P. M. Cunha, R. A. Sterry, E. P. B. Silva, J. N. Guenther, and M. C. Wiltbank. 2008. Altering the time of the second gonadotropin-releasing hormone injection and artificial insemination (AI) during Ovsynch affects pregnancies per AI in lactating dairy cows. J. Dairy Sci. 91:1044-1052.

Brusveen, D. J., A. H. Souza, and M. C. Wiltbank. 2009. Effects of additional prostaglandin $F_{2\alpha}$ and estradiol-17β during Ovsynch in lactating dairy cows. J. Dairy Sci. 92:1412-1422.

Caraviello, D. Z., K. A. Weigel, P. M. Fricke, M. C. Wiltbank, M. J. Florent, N. B. Cook, K. V. Nordlund, N. R. Zwald, and C. M. Rawson. 2006. Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. J. Dairy Sci. 89:4723-4735.

Cartmill, J. A., S. Z. El-Zarkouny, B. A. Hensley, G. C. Lamb, and J. S. Stevenson. 2001. Stage of cycle, incidence and timing of ovulation, and pregnancy rates in dairy cattle after three timed breeding protocols. J. Dairy Sci. 84:1051-1059.

Carvalho, P. D., M. J. Fuenzalida, A. Ricci, A. H. Souza, R. V. Barletta, M. C. Wiltbank, and P. M. Fricke. 2015. Modifications to Ovsynch improve fertility during resynchronization: Evaluation of presynchronization with GnRH 6 days before Ovsynch and addition of a second prostaglandin F2α treatment. J. Dairy Sci. 98:8741-8752.

Carvalho, P. D., V. G. Santos, J. O. Giordano, M. C. Wiltbank, and P. M. Fricke. 2018. Development of fertility programs to achieve high 21-day pregnancy rates in high-producing dairy cows. Theriogenology 114:165-172.

Chebel, R. C., M. J. Al-Hassan, P. M. Fricke, J. E. P. Santos, J. R. Lima, C. A. Martel, J. S. Stevenson, R. Garcia, and R. L. Ax. 2010. Supplementation of progesterone via internal drug release inserts during ovulation synchronization protocols in lactating dairy cows. J. Dairy Sci. 93:922-931.

Colazo, M. G. and D. J. Ambrose. 2015. Effect of initial GnRH and duration of progesterone insert treatment on the fertility of lactating dairy cows. Reprod. Domest. Anim. 50:497-504.

Cunha, A. P., J. N. Guenther, M. J. Maroney, J. O. Giordano, A. B. Nascimento, S. Bas, H. Ayers, and M. C. Wiltbank. 2008. Effects of high vs. low progesterone concentrations during Ovsynch on double ovulation rate and pregnancies per AI in high producing dairy cows. J. Dairy Sci. 91(E-Suppl 1):246 (Abstr.).

Dransfield, M. B. G., R. L. Nebel, R. E. Pearson, and L. D. Warnick. 1998. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. J. Dairy Sci. 81:1874-1882.

El-Zarkouny, S. Z., J. A. Cartmill, B. A. Hensley, and J. S. Stevenson. 2004. Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. J. Dairy Sci. 83:1024-1037.

Ferguson, J. D. and A. Skidmore. 2013. Reproductive performance in a select sample of dairy herds. J. Dairy Sci. 96:1269-1289.

Fricke, P. M. and M. C. Wiltbank. 1999. Effect of milk production on the incidence of double ovulation in dairy cows. Theriogenology 52:1133-1143.

Fricke, P. M., D. Z. Caraviello, K. A. Weigel, and M. L. Welle. 2003. Fertility of dairy cows after resynchronization of ovulation at three intervals after first timed insemination. J. Dairy Sci. 86:3941-3950.

Fricke, P. M., J. O. Giordano, A. Valenza, G. Lopes Jr., M. C. Amundson, and P. D. Carvalho. 2014. Reproductive performance of lactating dairy cows managed for first service using timed artificial insemination with or without detection of estrus using an activity monitoring system. J. Dairy Sci. 97:2771-2781.

Galvão, K. N., M. F. Sá Filho, and J. E. P. Santos. 2007. Reducing the interval from presynchronization to initiation of timed artificial insemination improves fertility in dairy cows. J. Dairy Sci. 90:4212-4218.

Giordano, J. O., M. C. Wiltbank, J. N. Guenther, R. Pawlisch, S. Bas, A. P. Cunha, and P. M. Fricke. 2012. Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch when compared to Ovsynch initiated 32 d after timed artificial insemination. J. Dairy Sci. 95:639-653.

Giordano, J. O., P. M. Fricke, S. Bas, R. Pawlisch, J. N. Guenther, A. B. Nascimento, and M. C. Wiltbank. 2013. Effect of increasing GnRH and $PGF_{2\alpha}$

dose during Double-Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows. Theriogenology 80:773-783.

Giordano, J. O., M. L. Stangaferro, R. Wijma, W. C. Chandler, and R. D. Watters. 2015. Reproductive performance of dairy cows managed with a program aimed at increasing insemination of cows in estrus based on increased physical activity and fertility of timed artificial inseminations. J. Dairy Sci. 98:2488-2501.

Giordano, J. O., M. J. Thomas, G. Catucuamba, M. D. Curler, R. Wijma, M. L. Stangaferro, and M. Masello. 2016. Effect of extending the interval from Presynch to initiation of Ovsynch in a Presynch-Ovsynch protocol on fertility of timed artificial insemination services in lactating dairy cows. J. Dairy Sci. 99:746-757.

Gümen, A., J. N. Guenther, and M. C. Wiltbank. 2003. Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. J. Dairy Sci. 86:3184-3194.

Gwazdauskas, F. C., R. L. Nebel, D. J. Sprecher, W. D. Whittier, and M. L. McGilliard. 1990. Effectiveness of rump-mounted devices and androgenized females for detection of estrus in dairy cattle. J. Dairy Sci. 73:2965-2970.

Haimerl, P., W. Heuwieser, and S. Arlt. 2018. Short communication: Meta-analysis on therapy of bovine endometritis with prostaglandin $F_{2\alpha}$ – an update. J. Dairy Sci. (in press; published online).

Hayashi, K. G., M. Matsui, T. Shimizu, N. Sudo, A. Sato, K. Shirasuna, M. Tetsuka, K. Kida, D. Schams, and A. Miyamoto. 2008. The absence of corpus luteum formation alters the endocrine profile and affects follicular development during the first follicular wave in cattle. Reproduction 136:787-797.

Herlihy, M. M., J. O. Giordano, A. H. Souza, H. Ayres, R. M. Ferreira, A. Keskin , A. B. Nascimento, J. N. Guenther, J. M. Gaska, S. J. Kacuba, M. A. Crowe, S. T. Butler, and M. C. Wiltbank. 2012. Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. J. Dairy Sci. 95:7003-7014.

Kiddy, C. A., D. S. Mitchell, D. J. Bolt, and H. W. Hawk. 1978. Detection of estrus-related odors in cows by trained dogs. Biol. Reprod. 19:389-395.

Lima, F. S., R. S. Bisinotto, E. S. Ribeiro, L. F. Greco, H. Ayres, M. G. Favoreto, M. R. Carvalho, K. N. Galvão, and J.E.P Santos. 2013. Effects of 1 or 2 treatments with prostaglandin $F_{2\alpha}$ on subclinical endometritis and fertility in lactating dairy cows inseminated by timed artificial insemination. J. Dairy Sci. 96: 6480–6488.

Lopes, G. Jr., J. O. Giordano, A. Valenza, M. M. Herlihy, M. C. Wiltbank, and P. M. Fricke. 2013. Effect of timing of initiation of resynchronization and presynchronization with gonadotropin-releasing hormone on fertility of resynchronized inseminations in lactating dairy cows. J. Dairy Sci. 96:3788-3798.

Lopez, H., L. D. Satter, and M. C. Wiltbank. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim. Reprod. Sci. 81:209-223.

Lopez, H., D. Z. Caraviello, L. D. Satter, P. M. Fricke, and M. C. Wiltbank. 2005. Relationship between level of milk production and multiple ovulations in lactating dairy cows. J. Dairy Sci. 88:2783-93.

López-Gatius, F., M. Lopez-Bejar, M. Fenech, and R. H. F. Hunter. 2005. Ovulation failure and double ovulation in dairy cattle: Risk factors and effects. Theriogenology 63:1298–1307.

López-Gatius, F. and R. H. F. Hunter. 2005. Spontaneous reduction of advanced twin embryos: its occurrence and clinical relevance in dairy cattle. Theriogenology 63:118-125.

Martins, J. P. N., D. Wang, N. Mu, G. F. Rossi, A. P. Martini, V. R. Martins, and J. R. Pursley. 2018. Level of circulating concentrations of progesterone during ovulatory follicle development affects timing of pregnancy loss in lactating dairy cows. J. Dairy Sci. 101:(in press, published online).

Moreira, F., R. L. de la Sota, T. Diaz, and W. W. Thatcher. 2000. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J. Anim. Sci. 78:1568-1576.

Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J. Dairy Sci. 84:1646-1659.

Nascimento, A. B, A. H. Souza, A. Keskin, R. Sartori, and M. C. Wiltbank. 2014. Lack of complete regression of the Day 5 corpus luteum after one or two doses of $PGF_{2\alpha}$ in nonlactating Holstein cows. Theriogenology 81:389-395.

Niles, A. M., A. E. Jones, P. D. Carvalho, and P. M. Fricke. 2017. Delaying administration of prostaglandin $F_{2\alpha}$ by 24 hours during a Double-Ovsynch protocol decreased fertility of lactating Holstein cows to timed artificial insemination. J. Dairy Sci. 100(Suppl 2):284.

Norman, H. D., J. R. Wright, S. M. Hubbard, R. H. Miller, and J. L. Hutchison. 2009. Reproductive status of Holstein and Jersey cows in the United States. J. Dairy Sci. 92:3517-3528.

Peralta, O. A., R. E. Pearson, and R. L. Nebel. 2005. Comparison of three estrus detection systems during summer in a large commercial dairy herd. Anim. Reprod. Sci. 87:59-72.

Portaluppi, M. A. and J. S. Stevenson. 2005. Pregnancy rates in lactating dairy cows after presynchronization of estrous cycles and variations of the Ovsynch protocol. J. Dairy Sci. 88:914-921.

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology 44:915-923.

Pursley, J. R., M. R. Kosorok, and M. C. Wiltbank. 1997. Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci. 80:301-306.

Pursley, J. R., R. W. Silcox, and M. C. Wiltbank. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. J. Dairy Sci. 81:2139-2144.

Remnant, J. G., M. J. Green, J. N. Huxley, and C. D. Hudson. 2015. Variation in the interservice intervals of dairy cows in the United Kingdom. J. Dairy Sci. 98:889-897.

Ricci, A., P. D. Carvalho, M. C. Amundson, and P. M. Fricke. 2017. Characterization of luteal dynamics in lactating Holstein cows for 32 days after synchronization of ovulation and timed artificial insemination. J. Dairy Sci. 100:9851-9860.

Santos, J. E. P., H. M. Rutigliano, and M. F. Sa Filho. 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Anim. Reprod. Sci. 110:207-221.

Santos, J. E. P., C. D. Narciso, F. Rivera, W. W. Thatcher, and R. C. Chebel. 2010. Effect of reducing the period of follicle dominance in a timed artificial insemination protocol on reproduction of dairy cows. J. Dairy Sci. 93:2976-2988.

Santos, V. G., P. D. Carvalho, C. Maia, B. Carneiro, A. Valenza, P. M. Crump, and P. M. Fricke. 2016. Adding a second prostaglandin $F_{2\alpha}$ treatment to but not reducing the duration of a PRID-Synch protocol increases fertility after resynchronization of ovulation in lactating Holstein cows. J. Dairy Sci. 99:3869-3879.

Santos, V. G., P. D. Carvalho, C. Maia, B. Carneiro, A. Valenza, and P. M. Fricke. 2017. Fertility of lactating Holstein cows submitted to a Double-Ovsynch protocol and timed artificial insemination versus artificial insemination after synchronization of estrus at a similar day in milk range. J. Dairy Sci. 100:8507-8517.

Silva, E., R. A. Sterry, D. Kolb, M. C. Wiltbank, and P. M. Fricke. 2007. Effect of pretreatment with prostaglandin F2α before resynchronization of ovulation on fertility of lactating Holstein cows. J. Dairy Sci. 90:5509-5517.

Souza, A. H., H. Ayres, R. M. Ferreira, and M. C. Wiltbank. 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology 70:208-215.

Sterry R. A., P. W. Jardon, and P. M. Fricke. 2007. Effect of timing of Cosynch on fertility of lactating Holstein cows after first postpartum and Resynch timed AI services. Theriogenology 67:1211-1216.

Stevenson, J. S., M. A. Portaluppi, and D. E. Tenhouse. 2007. Factors influencing upfront single- and multiple-ovulation incidence, progesterone, and luteolysis before a timed insemination resynchronization protocol. J. Dairy Sci. 90:5542-5551.

Stevenson, J. S., D. E. Tenhouse, R. L. Krisher, G. C. Lamb, C. R. Dahlen, J. R. Pursley, N. M. Bello, P. M. Fricke, M. C. Wiltbank, D. J. Brusveen, M. Burkhart, R. S. Youngquist, and H. A. Garverick. 2008. Detection of anovulation by heatmount detectors and transrectal ultrasonography before treatment with progesterone in a timed insemination protocol. J. Dairy Sci. 91:2901-2915.

Stevenson, J. S. and S. L. Pulley. 2012. Pregnancy per artificial insemination after presynchronizing estrous cycles with the Presynch-10 protocol or prostaglandin $F_{2\alpha}$ injection followed by gonadotropin-releasing hormone before Ovsynch-56 in 4 dairy herds of lactating dairy cows. J. Dairy Sci. 95:6513-6522.

Stevenson, J. S., S. L. Pulley, and H. I Mellieon Jr. 2012. Prostaglandin $F_{2\alpha}$ and gonadotropin-releasing hormone administration improve progesterone status, luteal number, and proportion of ovular and anovular dairy cows with corpora lutea before a timed artificial insemination program. J. Dairy Sci. 95:1831-1844.

Stevenson, J. S., S. L. Pulley, and S. L. Hill. 2014. Pregnancy outcomes after change in dose delivery of prostaglandin $F_{2\alpha}$ and time of gonadotropin-releasing hormone injection in a 5-day timed artificial insemination program in lactating dairy cows. J. Dairy Sci. 97:7586-7594.

Stevenson, J. S., J. A. Sauls, L. G. D. Mendonca, and B. E. Voelz. 2018. Dose frequency of prostaglandin $F_{2\alpha}$ administration to dairy cows exposed to presynchronization and either 5- or 7-day Ovsynch program durations: Ovulatory and luteolytic risks. J. Dairy Sci. 101:9575–9590.

Valenza, A., J. O. Giordano, G. Lopes Jr., L. Vincenti, M. C. Amundson, and P. M. Fricke. 2012. Assessment of an accelerometer system for detection of estrus and for treatment with gonadotropin-releasing hormone at the time of insemination in lactating dairy cows. J. Dairy Sci. 95:7115-7127.

Vasconcelos, J. L. M., R. W. Silcox, G. J. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 52:1067-1078.

Walker, W. L., R. L. Nebel, and M. L. McGilliard. 1996. Time of ovulation relative to mounting activity in dairy cattle. J. Dairy Sci. 79:1555-1561.

Wijma, R., M. M. Perez, M. Masello, M. L. Stangaferro, and J. O. Giordano. 2018. A resynchronization of ovulation program based on ovarian structures present at nonpregnancy diagnosis reduced time to pregnancy in lactating dairy cows. J. Dairy Sci. 101:1697-1707.

Wiltbank, M. C., P. M. Fricke, S. Sangritasvong, R. Sartori, and O. J. Ginther. 2000. Mechanisms that prevent and produce double ovulations in dairy cattle. J. Dairy Sci. 83:2998-3007.

Wiltbank, M. C., A. Gumen, and R. Sartori. 2002. Physiological classification of anovulatory conditions in dairy cattle. Theriogenology 57:21-52.

Wiltbank, M. C., G. M. Baez, F. Cochrane, R. V. Barletta, C. R. Trayford, and R. T. Joseph. 2015. Effect of a second treatment with prostaglanding $F_{2\alpha}$ during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. J. Dairy Sci. 98:8644-8654.