The evolution of fertility programs for lactating dairy cows

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Introduction

Synchronization protocols have been incorporated widely into reproductive management programs by most dairy farms in the US.[12,48] At first glance, it may seem that the newly released Reproductive Management Strategies for Dairy Cows protocol published by the Dairy Cattle Reproduction Council (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.[22] Newer fertility programs increase both service rate as well as pregnancies per artificial insemination (**P/AI**).[15] Each individual farm must implement a plan to submit cows for first AI and to identify non-pregnant 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 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 development of fertility programs for lactating dairy cows.

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.[12] Use of detection of estrus alone for submitting lactating dairy cows for first AI, however, generally results in poor reproductive performance because of 2 broad limitations associated with detection of estrus. The first limitation is with the human element (i.e., visual observation 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,[32] tail chalking, pedometry,[49] and radiotelemetry.[20,69] Dogs have even been trained to detect estrus-related odors in dairy cows.[36] 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 detection of estrus include the effect of high milk production on the duration of estrus,[39] ovulation failure after expression of estrus and ovulation without accompanying estrous behavior,[41,67] and anovular conditions in dairy cows.[72] Taken together, these human-related and cow-related issues substantially limit AI service rates and 21-d 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 3 sequential hormonal treatments are used to control ovarian function.[51] In the first field trial that evaluated the Ovsynch protocol for reproductive management,[52] lactating dairy cows managed 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 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. Because of the human-related 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.

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.[13,44,68] There are 2 broad categories of presynchronization strategies: 1) presynchronization using $PGF_{2\alpha}$, and 2) presynchronization that combines GnRH and $PGF_{2\alpha}$.

Presynchronization using $PGF_{2\alpha}$

The first presynchronization strategy tested used 2 $PGF_{2\alpha}$ treatments administered 14 d apart, with the second $PGF_{2\alpha}$ treatment preceding the first GnRH treatment of an Ovsynch protocol by 12 d[45] (**Presynch-Ovsynch**). 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. In fact, a recent meta-analysis of 3 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.[6] 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 2 $PGF_{2\alpha}$ treatments preceding the Ovsynch protocol were never intended to "clean the uterus", although this effect could 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 shown.[33] Indeed, administration of either 1 or 2 $PGF_{2\alpha}$ treatments before initiation of a Double-Ovsynch protocol had no effect on uterine health, P/AI, or maintenance of pregnancy in lactating Holstein cows.[37]

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. 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.[26] 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 better. 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 d vs a 14 d interval was compared[30] supporting the idea that inseminating cows to estrus during a Presynch-Ovsynch protocol negates the effect of presynchronization.[25] Further, 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 2 $PGF_{2\alpha}$ treatments of a Presynch-Ovsynch protocol, the Ovsynch protocol is initiated in a low progesterone (**P4**) environment resulting in fewer P/AI to TAI.[15] Because anovular cows represent 20% to 30% of cows submitted for first TAI,[1,56] presynchronization strategies using $PGF_{2\alpha}$ alone with or without inclusion of detection of estrus do not yield high P/AI to Timed AI.

Presynchronization that Combines 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 2 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) had more P/AI than cows submitted to a Presynch-Ovsynch protocol (50% vs 42%).[60] In a subsequent study, there was a treatment by parity interaction in which the Double-Ovsynch protocol increased P/AI for primiparous but not multiparous cows.[35] We now know this parity effect is due to incomplete luteal regression, particularly for multiparous cows.[73]

Presynchronization strategies have used a combination of GnRH and $PGF_{2\alpha}$ 6 to 7 d before G1 (i.e., **G6G** and **PG-3-G**.[3,63] 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.[63] 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.[15,64]

Synchronization Methods for TAI

Over time, there have been several variations of timing of treatments during an Ovsynch protocol that have been compared and used on dairy farms. 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 4 variations may work best for a given situation.

In the first published experiment using Ovsynch to hormonally synchronize ovulation,[57] lactating cows were submitted to TAI approximately 24 h after the last GnRH treatment of the protocol. All cows (n=20) ovulated to the last GnRH treatment of the Ovsynch protocol within 24 to 32 h, which is similar to the interval from the first standing event of estrus to ovulation of 27.6 h.[69] Thus, from a physiologic perspective, timing of ovulation is similar when comparing the interval from the first standing event of estrus or the last GnRH treatment of an Ovsynch protocol to ovulation.

To assess the effect of timing of AI relative to a synchronized ovulation, lactating dairy cows (n = 732) were randomly assigned to 5 treatments by stage of lactation and parity.[53] Ovulation was synchronized using an Ovsynch 48 protocol, and TAI was varied from 0, 8, 16, 24, or 32 h relative to G2. In this study, the 24 h treatment is equivalent to the **Ovsynch 48** protocol. Overall, cows in the 0, 8, 16, and 24 h treatments had more P/AI than cows in the 32 h treatment (Table 1). Thus, although no statistical difference in fertility was detected when TAI occurred from 0 to 24 h after the last GnRH treatment of the Ovsynch protocol, inseminating too late (i.e., at 32 hours) resulted in fewer P/AI.[53] 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 be physiologically relevant.

To further evaluate timing of AI relative to G2, a field trial was conducted to compare 2 variations of a Cosynch protocol (i.e., Cosynch 48 and Cosynch 72 compared in 2 earlier experiments),[50,61] in which TAI occurred concomitant to G2, with a variation of the Ovsynch protocol in which TAI occurred 16 h after G2.[10] This third treatment is now referred to as an **Ovsynch 56** protocol. Timing of AI in an Ovsynch 56 protocol is supported by the data in Table 1 in which the 16 h 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 h before ovulation.[20,69] 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 an 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 h interval from the $PGF_{2\alpha}$ treatment to the last GnRH treatment of the Ovsynch protocol and a 16 h interval from the last GnRH treatment to TAI. Despite the data in Table 2 supporting that an Ovsynch 56 protocol yields more P/AI, it is difficult for some farms to implement this timing of treatments due to the inconvenience or inability to handle cows in the afternoon. Most of these farms prefer the timing of the **Ovsynch 48** protocol or a **Cosynch 72** protocol. Thus, these Ovsynch variations are based on ease of implementation on farms rather than biology. Because of the extended interval between the last GnRH treatment of the Ovsynch protocol and TAI in a Cosynch 72 protocol, many cows will display estrus more than 12 h before scheduled TAI, thereby decreasing fertility to TAI.[10] Detection of estrus and AI from the $PGF_{2\alpha}$ treatment to the last GnRH treatment of a Cosynch 72 protocol can help to mitigate the decreased fertility to TAI when using this protocol variation.

The last option is a **5-day Cosynch** protocol in which the interval between G2 and the $PGF_{2\alpha}$ treatment is decreased from 7 (7-d protocol) to 5 (5-d protocol) d. The 5-day Cosynch

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 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 a row (P<0.10).

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 < 0.0).

protocol was first reported in a series of experiments in beef cows.[8] Although timing of AI after the $PGF_{2α}$ treatment differed between cows in the 7-d protocol than in the 5-d protocol, cows submitted to the 5-d protocol has more P/AI than cows submitted to the 7-d protocol in 2 experiments (80% vs 67%, respectively and 65% vs 56%, respectively). In 2010, the 5-d Ovsynch protocol was compared to a 7-d Cosynch 72 protocol in lactating Holstein cows.[57] In that study, cows submitted to the 5-d protocol received 2 $PGF_{2α}$ treatments, whereas cows submitted to the 7-d protocol received a single $PGF_{2α}$ treatment. Overall, cows in the 5-d protocol had more P/AI than cows in the 7-d protocol (38% vs 31%). The authors conducted an analysis to control for a difference in luteal regression rates between cows receiving 1 vs 2 $PGF_{2α}$ treatments by analyzing only cows with P4 < 1 ng/mL on the day of TAI, and cows submitted to the 5-d protocol had more P/AI than cows submitted to the 7-d protocol (39% vs 34%). The authors attributed this treatment effect to the decreased period of follicle dominance for cows in the 5-d Cosynch protocol. Colazo and Ambrose[18] also compared a 5-d Cosynch protocol with 2 $PGF_{2α}$ treatments to a 7-d Ovsynch protocol with 1 $PGF_{2α}$ 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 $PGF_{2α}$ treatment and the effect of decreasing the duration of the Ovsynch protocol from 7 to 5 d in a Resynch protocol.[58] Lactating Holstein cows (n = 821) were randomly assigned at a nonpregnancy diagnosis (d 0 = 32 d after AI) to 1 of 3 Resynch protocols: 1) 7D1PGF (GnRH, d 0; $PGF_{2α}$, d 7; GnRH, d 9.5); 2) 7D2PGF (GnRH, d 0; $PGF_{2α}$, d 7; $PGF_{2α}$, d 8; GnRH, d 9.5); and 3) 5D2PGF (GnRH, d 2; $PGF_{2α}$, d 7; $PGF_{2α}$, d 8; GnRH, d 9.5). All cows received an intravaginal P4 insert[a] at G1, which was removed at the first $PGF_{2α}$ treatment, and all cows received a TAI approximately 16 hours after G2. Overall, there was no effect of treatment on P/AI (Table 3). When these data were analyzed based on the presence or absence of a CL at G1, cows lacking a CL and receiving 2 $PGF_{2α}$ treatments had more (P=0.03) P/AI than cows receiving 1 $PGF_{2α}$ 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 $PGF_{2α}$ treatment to a Resynch protocol increased the proportion of cows 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-d 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.

Inclusion of a Second $PGF_{2α}$ Treatment 24 h after the First in Ovsynch Protocols

A major modification to Ovsynch protocols is the recommendation to include a second $PGF_{2α}$ treatment 24 h after the first in the 7 d Ovsynch protocol. Inclusion of a second $PGF_{2α}$ treatment is absolutely necessary for the 5-day Cosynch protocol due to a younger CL at the $PGF_{2α}$ treatment that fails to regress after a single $PGF_{2α}$ treatment.[46,65] Addition of a second $PGF_{2α}$ treatment is highly recommended for all of the 7-day protocols, particularly when used for first TAI after a presynchronization strategy that incorporates both GnRH and $PGF_{2α}$. The lack of complete luteal regression, particularly for multiparous cows which is addressed by the addition of the second $PGF_{2α}$ treatment, was in fact the rate limiting factor for fertility to TAI.[73] Indeed, submission of lactating Holstein cows to a Double-Ovsynch protocol and TAI for first insemination increased the percentage of cows inseminated within 7 d 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 day-in-milk range.[59]

Several experiments have been conducted to assess the addition of a second $PGF_{2α}$ treatment on luteal regression and P/AI.[2,11,66,73] A recent meta-analysis of data from these experiments was conducted with the primary objective to evaluate the effect of an additional $PGF_{2α}$ treatment during the Ovsynch protocol on luteal regression and P/AI.[7] The meta-analysis included 7 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. Includ-

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]**		
P/AI	**7D1PGF**	**7D2PGF**	**5D2PGF**	**T**	**C1**	**C2**
	-------------------- % (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 $PGF_{2α}$) and 7D2PGF + 5D2PGF (two $PGF_{2α}$) treatments; C2: preplanned contrast between 7D2PGF (7-d protocol) and 5D2PGF (5-d protocol) treatments.

ing a second $PGF_{2\alpha}$ treatment 24 h 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) 32 d after TAI using a fixed effects model. 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-d Ovsynch protocols is now recommended to increase fertility to TAI.

Although addition of a second $PGF_{2\alpha}$ treatment to Ovsynch protocols dramatically 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 US: 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 appear to perform as well as 2 25 mg dinoprost treatments administered 24 h apart for first[66] or Resynch[2] TAI. Increasing the dose of cloprostenol from 500 to 750 µg increased the rate of luteal regression primarily in multiparous cows, but tended to increase fertility (P=0.05) only at the pregnancy diagnosis 39 days after TAI.[28] Finally, delaying a single dinoprost treatment by 24 h (i.e., from day 7 to day 8 of the protocol) without adjusting G2 and TAI decreased luteal regression and P/AI.[47] 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 h after the first to both 7 d and 5 d Ovsynch protocols should be followed.

Resynchronization Programs

There are now 2 major options for Resynchronization programs based on timing of nonpregnancy diagnosis and initiation of the Resynch protocol. Although both 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. Nonetheless, including detection of estrus after an initial AI can increase 21-d pregnancy rates by increasing the AI service rate. Farm managers should keep in mind that they must manage 2 reproductive management systems in this scenario; 1 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 re-inseminating 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.[29] Second, estrous cycle duration varies widely with a high degree of variability among individual cows.[54] 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.[55] Because of these issues with nonpregnant cows returning to estrus, implementation of a Resynch strategy is critical for achieving high 21-d pregnancy rates.

Timing of Pregnancy Diagnosis and Initiation of Resynch

In the first strategy for Resynch, nonpregnancy diagnosis is conducted before initiation of the Resynch protocol, whereas in the second strategy, the first GnRH treatment of a Resynch protocol is initiated 7 d before nonpregnancy diagnosis. Choosing between these 2 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.[9] 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 1 week due to 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 high proportion of cows diagnosed pregnant at the herd check, and these cows are unnecessarily treated with GnRH. By contrast, an advantage of administering G1 before pregnancy diagnosis is that TAI occurs 1 week earlier. Overall, P/AI did not differ between cows submitted to a Resynch protocol 32 or 39 d after AI,[38] 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).

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 CL) at the $PGF_{2\alpha}$ treatment of the Ovsynch protocol.[15] 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.[24] Assuming an estrous cycle duration of 21 to 23 d, administering G1 32 d after AI should correspond to initiating the Resynch protocol around day 6 to 14 of the estrous cycle, a stage of the estrous cycle when a dominant follicle and a CL with mid-level P4 concentrations should be present. Cows identified not pregnant 32 d after AI with a CL at G1 have more P/AI than cows without a CL.[27,38] 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.[24,29] When cows were synchronized for first TAI and P4 profiles and CL diameter was measured until a pregnancy diagnosis 32 d later, 19% of cows diagnosed not pregnant lacked a CL > 10 mm in diameter.[55] 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 h after the first into a Resynch protocol increases P/AI for cows initiating Resynch in a low-P4 environment.[14]

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 d had more P/AI at first as well as Resynch TAI.[4,5,17] 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.[70] 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 2 $PGF_{2\alpha}$ treatments 24 h 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 3 Resynch treatments. The authors concluded that the shorter Resynch program decreased time to pregnancy because of a decrease of the interval between AI services for nonpregnant cows with a CL and more P/AI in nonpregnant cows lacking a CL.[70] 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.[15] 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 h after the first. Nonpregnant cows lacking a CL restart an Ovsynch 56 protocol that includes a second $PGF_{2\alpha}$ treatment 24 h after the first (i.e., GGPPG) as described by Carvalho et al.[14] Intravaginal P4 inserts (1 per cow) are included within 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.[4,5]

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

Low P4 during growth of an ovulatory follicle is associated an increased incidence of double ovulation.[71] 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.[34,62] All dairy cows experience a low P4 environment during the postpartum anovular period from calving to first ovulation. Double ovulation rate after a spontaneous estrus was greater for anovular cows (i.e., low P4) than for cycling cows.[40] 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.[31] Thus, the first postpartum ovulation results in a high double ovulation rate due to 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 randomly assigned to 2 presynchronization protocols that manipulated cows into either a high or a low P4 environment during an Ovsynch protocol (Table 4).[19] Cows in the high P4 treatment were submitted to a Double-Ovsynch protocol[60] 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 the last GnRH treatment of the Ovsynch protocol 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,

Table 4. Effect of progesterone (P4) during growth of the preovulatory follicle on incidence of double ovulation in Holstein dairy cows.*

Item	Low P4 (n = 259)	High P4 (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

*Adapted from Cunha et al, 2008.

Table 5. Effect of progesterone (P4) 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.*

Item	Low P4 (n = 40)	High P4 (n = 40)	*P*-value
Follicle size at G2 (mm)	16.4 ± 0.5	14.8 ± 0.3	<0.01
Double ovulations (%)	33 (13/40)	10 (4/40)	<0.01
P/AI at 32 d (%)	53 (21/40)	45 (18/40)	0.97
Twins at 32 d (%)	29 (6/21)	0 (0/18)	<0.01

*Carvalho et al, 2019.

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.

It is important to note that the study by Cunha et al was conducted before the second $PGF_{2\alpha}$ treatment was included in the Ovsynch protocol.[19] 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, cows in the low P4 treatment had high double ovulation rates but low conception rates due to incomplete luteal regression.[19] For cows that initiate an Ovsynch protocol in a low-P4 environment, if you fix the luteal regression problem by adding a second $PGF_{2\alpha}$ treatment, P/AI could increase dramatically due to increased double ovulations[23] followed by increased pregnancy losses for cows that conceive unilateral twins,[42] 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 h after the first, and were randomly assigned to receive 25 mg $PGF_{2\alpha}$ 1 d after the first GnRH treatment of the breeding Ovsynch protocol 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.[16] 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.[43] 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%.[43]

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 initiates the protocol in a low-P4 environment. This scenario also leads to increased pregnancy losses due to bilateral twins,[43] and may explain a significant proportion of pregnancy losses that occur in dairy herds). There are 2 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.[25] This scenario can be avoided by using a presynchronization strategy that combines both GnRH and $PGF_{2\alpha}$, because these presynchronization strategies set up a high 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 double ovulation rate.

Conclusion

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, and 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/.

Endnotes

[a] PRID Delta; Ceva Santé Animale, Libourne, France

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